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STUDIES ON THE DESTRUCTION OF VITAMIN C IN THE BOILING OF MILK

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Introduction

MILK always has been, and will undoubtedly continue to be, the most important and indispensable single food of man. Because of its importance in the dietary of the infant and growing child as well as the adult (1) its nutritive properties have been studied more than those of any other food. The present investigation is concerned with the degree of destruction of vitamin C during the boiling of this food. This is important to the public health, as the pediatrician usually feeds boiled cow's milk, when necessary to supplement or supplant breast feeding, for the purpose of obtaining satisfactory size of the particles of curd in the infant's stomach (2). It has been found that this is a most effective means of controlling the incidence of those alimentary infections known colloquially as "Summer Complaint" (3). With the development of the practice of boiling milk there has been considerable evidence advanced to show that city milk is not as rich in antiscorbutic properties as some other foods (1, 4). Although we would not care to depreciate the use of other foods containing high potencies of vitamin C, we would not pass lightly over the importance of milk as a source of this antiscorbutic vitamin, especially in view of its early reputation as the sole successful source of an antiscorbutic agent for infants.

Vitamin C was selected for study in our cooking experiments, since this appears to be the most unstable vitamin. The effects are clearly

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differentiated by the marked relative (if not absolute) difference in susceptibility of the rat and the guinea pig to its absence. Moreover, changes in academic thought are less likely to make abruptly obsolete the results of studies based upon such differences between two species of animals.

Hess and Fish (5) state that milk boiled for a short period does not induce scurvy so readily as pasteurized milk, although both practices lower the vitamin C content. Home pasteurization is less deleterious than industrial pasteurization, since the latter involves especially problems of aging. Evaporated milk ordinarily contains no vitamin C (6, 7) though sweetened condensed milk may contain it (4). Dried milk powder may contain it even in considerable amounts (4, 8). Hess (4) states that a pint of ordinary city milk a day per nursing infant would generally suffice, and this is within the regular feeding regimen. The fact that the infant at present is generally left to get his vitamin by "probability and chance," under conditions which are adverse to him, is sufficient to emphasize the marked possibilities of developing a milk supply sufficient in vitamin C for maintainence of health.

The fundamental factors operating to affect the vitamin C content of milk are; feeding practices of dairymen (9, 10, 11, 12), and temperature and exposure of the milk to oxygenation after it is produced (4), namely, storage practices, cooling, straining and pasteurization and repasteurization. Under the caption of oxidation *per se*, is also included the effect of the container coming in contact with the milk, because any effect this might have would probably be catalytic oxidation. Copper has been shown by Hess (13) to be particularly conducive to destroying vitamin C during the pasteurization process. Bright copper seems to be less liable to produce off-taste in milk when it is used immediately after cleaning (14), presumably since there is less oxide on the surface at this time. McCollum, Simmons, and Becker (15) have shown that ferrous sulfate added to the prepared type of rat food is conducive to the destruction of vitamin A.

The catalytic action of metals, especially those possessing more than one valency, is too well known in the field of chemistry to demand comment (16). Mellor (17) states that aluminum possesses only one valency. Moreover Waters (18) has shown that aluminum and zinc are inactive and suitable for containers for transformer oils which are subject to oxidation whereas some other metals are less suitable. It would appear possible, therefore, that aluminum might be shown to possess a distinct advantage as regards the absence of any oxidative effect on vitamin C. Its qualities in other respects for dairy equipment and comparisons with other metals are fully discussed by Hunziker, Cordes and Nissen (19).

General Discussion of Methods of Assaying the Antiscorbutic Vitamin

In the present state of our knowledge, vitamins are known only by their effects on animals. Their chemical isolation has not yet been accomplished and recent work on vitamin D and ultra-violet light illustrates that it is a debatable question whether they are entities or states of matter. Man, monkey, and the guinea pig, are the only animals, with the possible exception of the domestic pig, known to be susceptible to scurvy (4). Guinea pigs are used practically exclusively for studies on vitamin C. Whether vitamin C be simple or a complex mixture, this term refers merely to a difference in the response of rats and guinea pigs to the same particular diet.

Several methods of assaying vitamin C have been proposed. Ordinarily comments upon these should be confined to a communication primarily on methods, except for the fact that a misconception exists in the minds of some as to the accuracy and the full meaning of an assay. At present the methods are at best "crude" (4), and the information obtained is of relative value only. The fundamental fact underlying this situation is that it is impossible to evaluate exactly degrees of effect, as one would determine them by weight or by titration. The best that can be done in a physiological assay is to manipulate dosages until a fairly general and easily recognizable effect is obtained (20). Moreover, animals may vary considerably in initial vigor as well as in response, even when care is taken to obtain as much equality as possible. Furthermore, when one considers that the line of distinction between the state of health and the state of disease, as well as that attribute known as "physiological reserve" (which comes into play in time of stress), are still fruitful subjects for debate, we would expect that the scurvy assay would necessarily be one of the topics involved.

The designation of a method of assay as the "minimum protective dose" method implies that we have reached the stage where we readily recognize complete protection. This is not so, because of our inability to distinguish between the real protection, involving the optimum dose, and apparent protection, which merely is the stage of imminent or incipient scurvy. It has been reported that 15 cc. of milk protected one out of three animals, 30 cc. protected two out of three, and 50 cc. protected all (10). By common consent any one of these doses could be taken to mean the "minimum protective dose" according to whether we mean protecting just a few, about 50 per cent, or practically all animals. Three cc. of fresh canned tomato juice is said to be the "minimum protective dose," but

all animals fed this dose, and so reported, had mild or very mild scurvy at some stage (21, 22). The terms as used experimentally, therefore, may have different meanings and these possible differences in the points of view should be considered.

The short and so-called "minimum curative dose" method which is used for tests on fresh fruits, etc., in season, has the same objections. It implies to the casual observer, who is not familiar with scurvy experimentation, that we can produce standard and exact degrees of scurvy, and that complete curing is possible. Both assumptions are far from proven. Although the method is useful we should appreciate the relative meaning of the results.

Sherman and his pupils (21, 22, 23) have elaborated a numerical scoring system for the statistical treatment of pathological findings. Interpolations (23) have been suggested as well as extrapolations (12). We have also used this system merely as a shorthand method of recording within limits the degree (1, 2 or 3, or mild, moderate, or severe) of the lesion found in various locations. Undoubtedly some system which will summarize pathological findings in a satisfactory form is much needed. We have taken the average of the individual scores of our animals in the same groups, but for the present would prefer to limit the interpretation from the scoring of pathological lesions and their statistical treatment, to only those degrees of difference which we might be able to bring out by the more laborious method of considering each group of individuals separately, and by their general distinction from individuals in other groups.

Another factor enters, namely, in the indirect or "secondary" effects of the vitamins. In ophthalmia, due to lack of vitamin A, infection undoubtedly plays the dominant rôle in the outward symptom. It has recently been suggested that the outward manifestations of beriberi (24) are due to an infection, which is made possible by lack of the antineuritic vitamin. There have been numerous theories as to the infectious nature of scurvy (4). There is no doubt that scorbutic animals are infected. So long as we rely on morbid states and conditions as revealed clinically and pathologically, it does not seem that we will avoid entirely assaying the chances of an animal becoming infected and acting as a host, rather than the primary effect of an absence of vitamin on the animal's own functions. This situation illustrates the more sound logic in using the capacity for growth or other physiologic attributes for assaying vitamins. The lamentable situation, however, is that growth curves in scurvy experiments have not generally been found to be suitable for use, probably on account of the genetic heterozygosity in ordinary stock animals, as well

as latent and chronic disease in commercial guinea pigs. The alien articles of diet, such as milk, which we attempt to test upon this species of animals, together with unnatural methods of administration, also influence the well being of many animals. Undoubtedly progress in the assay of vitamin C may be expected as soon as these mitigating circumstances are improved or eliminated.

All investigators working within the last decade meet on common ground in that they have, if possible, employed growth curves, food intake data, clinical appearances and gross post-mortem findings. Histological findings might help bridge the width of the gap between "minimum protective" and optimum dosages, but would probably not result in any greater sharpness of the decision. For the present the greatest hope of security is to be found in collection of the maximum amount of data possible and the test of conclusions in as many ways as possible. The statistical manner in which investigators often have to treat their data and the dynamic way in which growth, appetite, symptoms, and pathological lesions progress and regress, suggest that the investigator will eventually have to readjust himself and come to analyze nature's manifestations entirely from his own point of view.

Experimental Procedure

Commercial guinea pigs were used. These were obtained from the minimum number of breeders possible. A short preliminary period in the laboratory permitted an adjustment to cage life and the elimination of poor eaters, as well as poor consumers of milk. Animals were kept in tinned iron, woven wire cages having 2–1/2 meshes to the inch. Trays or pans, as well as the false bottom screen, were of tinned (dipped) iron. The cages were completely demountable. Ammoniacal vapors attacked these cages at "pinholes" in the tin plating, but they were entirely resistant to alkaline cresol disinfectant. Opal ointment jars of four ounce capacity served as feeding cups, and were wired into a corner of the cage. Water was given by an inverted bottle with a glass tube which projected into the inside of the cage. Animals were weighed, food changed, and water bottles, trays and screens, sterilized every third day. These duties, as well as the feeding of milk, were carried out on Sundays and holidays as regularly as on week days.

Diet. We used the diet of Kenny (23) and MacLeod (12), with allowances for differences in the size of the doses of liquid milk fed by varying the butter fat and the skim milk powder contents. The value of the assumption that the amount of milk solids would thereby become equal

for all animals was not completely realized, as the appetite was usually disproportionately restricted in the animals receiving the larger doses of liquid milk. The composition of the diet and dosages of milk are given in Table I.

TABLE I

COMPOSITION OF DIETS IN TERMS OF ESTIMATED AMOUNTS OF ONE DAY'S RATION

Dose of Milk fed	62.50 cc.	50 cc.	40 cc.	32 cc.	25 cc.	20 cc.	0. cc.[1]
	gms.	gms.	gms.	gms.	gms.	gms.	gms.
Heated skim milk powder	0.0	1.124	2.1	2.88	3.6	4.15	6.0
Bran	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Rolled oats	7.8	7.8	7.8	7.8	7.8	7.8	7.8
Butter fat	0.0	0.376	0.7	0.92	1.2	1.35	2.0
Sodium chloride	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Total dry diet	12.0	13.5	14.8	15.8	16.8	17.5	20
Protein in dry diet	1.88	2.27	2.68	2.97	3.24	3.45	4.15
Total crude protein per day[2]	4.05	4.01	4.07	4.08	4.11	4.14	4.18

[1] Full basal diet, (See Tables II and IV).

[2] Assuming that the ration herein calculated was completely eaten and including the protein in liquid milk fed.

Our first experience during a preliminary experiment with heated skim milk powder revealed that the described method for its devitaminization was not adequate for our particular sample of material. We next made arrangements with Mr. R.S. Fleming of the Merrell-Soule Company, Syracuse, New York, to heat a large batch of powder in an industrial roaster at 110° C for four hours, with constant stirring and continuous aeration, believing that this would be conducive to securing a more certainly devitaminized product, on account of continuous mixing and good exposure to air.

A series of 10 negative control experiments was run on this batch of heated skim milk powder before the regular experiment was started. (See Table II). Since the average was one day less than that in the series of LaMer which we have summarized in Table II, and since two of our contemporaries who are fully familiar with this heated skim milk powder diet told us that occasionally a negative control animal may live longer, than the 34 day period, on a diet completely free from vitamin C, we assumed that the diet was free from vitamin C. Later we became sus-

picious of the sufficiency of the absolute value of this negative control test, and ran another series of seven tests which also are shown in Table II.

We also made a more careful and critical review of the literature and found that as regards scurvy-producing diets, investigators were grouped into two camps, one claiming that their particular diet was free and the other realizing the inadequacy of criteria for judging the test and being satisfied with a diet which would give evidence of producing acute scurvy and death in relatively short experimental periods. The evidence is entirely in favor of the latter group because, as far as any proof is concerned, there is none which shows how long guinea pigs should live; as no diet had been proved to be free from vitamin C, nor has the influence of previous diet upon longevity been studied. Reference to Table II will reveal that the time of heating skim milk powder has been increased from 2 to 4 hours by succeeding observers with the result that longevity has decreased. We do not interpret this as any evidence that the diets used by LaMer (21) and Sherman, LaMer and Campbell (22) or by Kenny (23) contained vitamin C, but rather cite this possible coincidence and conclusion to indicate the impossibility of being absolutely certain one way or another.

As a result of this dilemma, we ran a series of negative controls on a diet of oats, bran, butter fat and salts. This diet, plus tomato juice, we have shown also to be adequate for nutrition. We also ran a series in which 2.2 cc. of the raw milk used in this investigation, diluted to 10 cc. with water, was fed daily for 27 days (being discontinued then on account of causing some aspiration pneumonia in weak and scorbutic animals) with the result that 7 satisfactory experiments lasted 33 to 40 days, or an average of 35.7 days. Since this is 5.5 per cent of the certain minimum protective dose herein reported, we reached the conclusion that there was certainly not more than 2.25 per cent of the minimum protective dose of vitamin C in a day's ration of our full skim milk powder basal diet. Actually this error is still further reduced, as consultation of the Table will reveal that the amount of heated skim milk powder which we fed was from two-thirds of the amount in the full basal diet to no milk powder whatsoever. Consequently this magnitude of error is entirely within the accuracy of the method of assay of antiscorbutic vitamin and need be given no further concern. We have, in presenting these data, gone on the pure assumption, as all others before us, that oats and bran contain no vitamin C.

It should be noted also that our technique differed from that of others using the skim milk powder diet, in that we used one large lot of powder;

TABLE II

SUMMARY OF NEGATIVE CONTROL EXPERIMENTS

Observer or Source	Number of Experiments	Average Initial Weight	Average Maximum Weight	Average Final Weight	Average Loss in Weight	Duration of Experiment		Score	Time of Heating Skim Milk Powder
						Average	Range		
		gms.	gms.	gms.	per cent	days	days		Hours
Series I. This investigation	10	311	334	197	37	29.1	23–36	16.8	4
Series II. This investigation	7	333	372	218	35	32.1	23–37	18.3	4
Total or Average. Series I and II.	17	320	349	206	36	30.3	23–37	17.3	4
LaMer (21) Sherman	10	337	372	204	40	30.0	26–34	19.1	2
LaMer and Campbell (22)	15	329	352	202	39				
Kenny (24)	10	353	—	214	39	28.6	21–30	14.9	?
MacLeod (12)	9	—	—	—	—	22.6	19–24	17.1	4

whereas the customary procedure in its preparation is to heat the powder from time to time as needed, thereby placing the burden of experimental credulity upon the method of heating rather than upon the food material itself. The usual custom of testing the diet is to run one to three animals simultaneously, and about three sets during a regular 90-day experiment. Our results would indicate the importance of more numerous tests performed simultaneously, which is another reason for preferring a large, single lot of each dietary constituent.

Two lots of rolled oats, two of bran, and two of butter were used. The first lots were tested in the first series of negative control experiments reported in Table II and the second lots in the second series, respectively. We regretted this procedure but were unable to avoid it as the supply of the first lots of dietary ingredients gave out.

Quality and Dosages of Milk. Our preliminary experiments on city delivered certified milk from cows fed on the pasture revealed that 50 cc. were insufficient to protect guinea pigs from scurvy. Believing that this was evidence of destruction through aging, probably as a result of oxidation, we cast about for a means of obtaining milk as soon as possible after milking. We were able to make arrangements with the Hermes-Groves Dairy Company, now a unit of the Liberty Milk Company, for obtaining from their Braeburn Dairy daily at 9 A.M. certified raw milk which had been milked at about 4 A.M. the same morning. The milk was mixed milk obtained from the same three cows for about the first two months of the experiment, but later (owing to three or four accidents, such as fire, reorganization, change in personnel etc.) some changes in animals had to be made. We therefore lost this continuous phase of our experiment, although each cow used throughout our experiment was on 35 pounds of green corn ensilage daily and the regular feeding regimen based upon quantity of milk produced by each cow.

Three groups of experiments were started; raw milk, milk boiled in aluminum, and milk boiled in glass. Dosages of 62.5, 50 and 40 cc. were started in the two boiled milk series and 50, 40 and 32 cc. in the raw series. These dosages stand in relation of 25 per cent increases or 20 per cent decreases. Some replacements were necessary early owing to some acute pneumonia, as well as to some animals, especially in the larger dosage groups, going off the diet. Within six weeks of starting the original experiments, two lower dosage groups had been started in each series, (25 and 20 cc. raw milk, and 32 and 25 cc. dosage groups on boiled milks) owing to the general failure of scurvy to appear in the animals which had been first started.

Administration of Milk. Milk was administered by a pipette in preference to feeding in cups, to avoid losses from spilling and to eliminate variations in the willingness of the animal to consume a given amount of milk. This was also done to eliminate variations in the time of consumption which might have affected its vitamin content, especially at room temperature, as well as the consumption of cream layers. MacLeod (12) also fed milk with a pipette. All others have employed cup feeding, at least in part.

It can not be stated that feeding milk by pipette is free from objections. MacLeod found 50 cc. the limit. We accomplished feeding 62.5 cc. per 300 grams body weight but not without considerable effort, a high percentage of animals going off the diet. We feel that both these dosages are too large for a most satisfactory and easily conducted series. The difficulty came apparently from too much swallowed air, the nitrogen probably not being absorbed from the intestine and not being readily propelled along in the larger amounts. Animals on high dosages were occasionally quite "bloated." The condition is probably similar to colic in the nursing infant. Every effort was made to save those responding poorly at the start, but we feel rather that efforts should be confined to starting a sufficient number at the beginning to permit elimination of those which show any tendency to restrict their food intake too much.

The capacity of the stomach of guinea pigs would seem to be at least 25 cc. per 300 gms. body weight, but we have limited the feedings except in special cases to 20 cc. and less. Postmortem examination of animals some time after feeding large doses revealed that functioning of the stomach was grossly perfect. Whey tended to leave rather soon while curds were retained. Hess states that it requires 80 to 100 cc. of milk a day to protect guinea pigs, (weight of animals not stated) and we infer that very few will consume this amount by cup, even when proffered over a long period of time. After all things have been said, we believe that feeding by pipette is nevertheless the more desirable, since it is conducive to the greater accuracy and certainty, but it is expensive and time consuming. In practice it amounts, in either method of feeding, to the obtaining of a sufficient number of satisfactory animals. The rate of feeding milk by hand is about 80 cc. per hour under average conditions.

The milk was measured in 25 cc. graduates. Allowances were made by weight for any losses in feeding by catching that which was slobbered or spat out, on a cloth and by wiping off the chin. After several days most animals learned to drink without loss. The pipettes were of hard glass, of about 7 cc. capacity, and had a narrow, practically cylindrical tip

about one half inch long. A commercial rubber bulb of ½ ounce capacity was used at the upper end. Care was taken not to trap milk in the rubber bulb. The glassware and rubber bulbs were sterilized each day in alkaline hypochlorite solution, and also after feeding any pneumonic pigs which we may have tried to save. Animals with colds or pneumonia were isolated from the healthy ones and fed separately.

Boiling and Care of the Milk. It required an average of 19.3 (±2.2) minutes to bring milk to the boiling point in the aluminum sauce pan and 26.9 (±4.2) minutes in the glass beakers. (The standard deviations are given in parentheses). The rate was the natural one for the aluminum, but it was pushed in the case of the glass as rapidly as possible, without securing a scorched taste, in order to bring the time for glass as nearly as possible to that for aluminum. Light boiling was continued 5 minutes, which is the time used by Hess (4) and also given by Brennemann (3). Some pediatricians use less time. Three quarts of milk were boiled at one time, which represents more than a probable maximum in the home or hospital.

As soon as practicable the hot milk was poured through a cheese cloth in a glass funnel into pint bottles and stored in an electric refrigerator kept below the freezing temperature. No milk froze up to the time of the last feeding. The milk was at room temperature when fed, being taken as needed out of a pint bottle, exposed to the air but covered with a cap. Equalization of any errors introduced at this stage occurred, since the order of feeding differed from day to day. The utensils were thoroughly washed daily, the aluminum pan being polished with steel wool. The pint milk bottles were sterilized daily in the oven of a gas range. The age of the milk fed, considering time in transit and averaging early and late feeding times, was practically always under 10 to 12 hours.

Discussion of Experimental Results

There are given in Table III the analytical results from the boiling of water and milk in aluminum. The amounts of aluminum taken up are to be regarded as within the limits of the unavoidable. Such amounts could easily come from sources other than aluminum cooking utensils such as dust contamination as well as hydrolysis of rock and soil on weathering in water sheds and in rivers. There are also given in the table the results of analyses performed upon whole milk powder. Since liquid milk is one-eighth solids, dividing these results by 8 would bring the concentration of aluminum in remade whole milk to 0.4 to 0.5 parts per million. These results are therefore in agreement with those of other milks examined.

TABLE III

ALUMINUM CONTENT OF WATER AND OF MILK BOILED IN ALUMINUM

Material Tested	Time in Bringing to Boiling	Duration of	Amount taken		Aluminum		Remarks
			Boiling	One Analysis	Before	After	
	minutes	minutes	cc.	cc.	parts per million	parts per million	
Tap water	—	1	1000	250	0.6	0.8	From Pittsburgh City Main. Pan #19, 2-S Metal.
Certified milk	19	5	3000	200	0.5	0.6	Fresh, 6 hours old. Pan #19, 2-S Metal.
Store milk	60	30	—[1]	250	0.6	1.0	Age of milk unknown. Stirred with aluminum spoon intermittently. Pan #1, 3-S Metal.
Commercial whole milk powder							
Lot A	—	—	—	—	3.2	—	
" B	—	—	—	—	3.0	—	
" C	—	—	—	—	4.0	—	
" D	—	—	—	—	4.0[2]	—	

[1] More than 1000 cc.

[2] Preliminary determination.

The analyses were performed colorimetrically with the dye aurin tricarboxylic acid. Iron was separated by the cupferron method. Full recoveries of added aluminum were obtained. The expression of the results is in round numbers on account of an experimental error of about 10 per cent, as well as the concentration of aluminum being the least that is susceptible to handling conveniently.

There is given in Table IV a summary of the animals receiving the full basal diet plus 15 grams of fresh spinach daily. These animals were fed for 89 days or more, and were for the purpose of furnishing control autopsy material. They were not for the purpose of positive controls in the true sense, since these latter experimental animals are properly found at the top of a scurvy series. Controls on the basal diet plus 15 grams of spinach daily should, however, be identical with those which one would obtain at the top of an experimental series, if several conditions did not enter. We do not know at present that the skim milk powder diet is optimum in all respects. It is known, but not well known, that animals receiving protective doses of vitamin C from different scources, but the same basal diet, may differ in growth[1].

The growth of these animals was satisfactory, because others were eliminated early that failed to react so. Since there were fewer animals than the number of days on which animals of the regular experiment were killed for autopsy, we dispensed with the service of an autopsy standard on several days, but held animals in readiness for use if needed. The condition of these autopsy controls was good except that one gave evidence of having had a respiratory impediment, probably pneumonia, as judged by rotated and somewhat angulated ribs. Broadening of the ribs, especially the junctions, was found in two; but the relations of the finer details were absolutely normal to the naked eye. Our early suspicion that broadening of the ribs was of scorbutic origin, possibly being a reaction to less than the optimum amounts of antiscorbutic agent, was neither confirmed or disproven. The condition was not infrequently found in animals concerning which no suspicion of scurvy was ever had.

Since we used commercial animals, we realized the necessity of controls for estimating their quality. Twenty-six animals became available early in the preliminary period in which the response to milk was being studied. These were killed and autopsied and found to be free of scurvy. Later four more were obtained, remaining from a group of substitutes, and were likewise free of scurvy. These animals, however, showed some pneumonic conditions which we attributed in part to accidents during the preliminary

[1] Personal communication from Prof. W. H. Eddy.

TABLE IV

SUMMARY OF RECORDS OF ANIMALS FOR AUTOPSY CONTROL

Animal Number	Original Weight gm.	Final Weight gm.	Gain gm.	Days Duration of Experiments	Gain per Day gm.	Total Food Eaten gm.	Food per day gm.	Gain per gm. Food Eaten gm.	Note on Pathological Examination
257	323	576	253	113	2.24	2073	18.34	.122	No scurvy
312	348	573	225	90	2.5	1909	21.21	.118	No scurvy
304	342	733	391	90	4.33	2130	23.67	.185	No scurvy
327	295	575	280	89	3.15	1753	19.70	.160	No scurvy
334	278	450	172	117	1.47	1963	16.77	.087	No scurvy
395	325	658	333	102	3.26	1986	19.47	.168	No scurvy
404	314	700	386	112	3.45	2504	22.36	.154	No scurvy
407	328	567	239	112	2.13	2203	19.67	.108	No scurvy
421	318	726	408	113	3.61	2805	24.82	.145	No scurvy certain. Nervous pig throughout experiment.
571	373	746	373	89	4.19	2360	26.52	.158	No scurvy
576	373	578	205	90	2.28	1805	20.06	.114	No scurvy
Average	329	626	297	102	2.96	2135	21.14	.138	

period. There was also some chronic lung disease more or less of which is always found in commercial animals. Later 50 animals came to autopsy, which included members from all lots of animals which we purchased; two of these animals were regarded as doubtful but given zero scurvy scores, and two were given scurvy scores of "1" each (maximum score = 27).

For all animals the chances are then two in seventy, or about 3 per cent, of meeting traces of scurvy existing previous to entering the laboratory. We would call attention to the general absence of any control of this sort having been regularly exercised by investigators, especially those using commercial animals, and would certainly feel that this source of error should be carefully controlled in the future. Cavenaugh, Dutcher, and Hall (8) have called attention to the necessity of examining animals for signs and symptoms of scurvy before use. All investigators attempt a short preliminary period of feeding before starting the regular experiment. It is questionable, however, whether even these precautions will suffice, particularly if we employ histological evidence, as the lesions of scurvy have not been proved to heal by resolution or without leaving marks of their previous existence (25). It is probable that the most satisfactory solution of this matter will come in breeding animals in the laboratory after the methods of scurvy experimentation become sufficiently facile to permit the employment of smaller numbers than are at present required.

A summary of the clinical and pathological scorbutic findings is given in Tables V, VI and VII. The data obtained from animals surviving the full experimental period are given separately from those obtained from animals which died previous to the expiration of 90 days, but after the 45th day. The experiment passed through the vicissitudes of an acute pneumonia epidemic at the time of the general human epidemic of December, 1928. By reference to Tables VIII, IX, and X, those animals showing no pathological lesions whatsoever, other than those of scurvy, or simulating scurvy, can be identified, so one is privileged to make a comparison on the basis of only the entirely healthy animals if desired.

The presentation of the clinical data is self explanatory, with the exception of the doubtful column. This column lists those cases in which we deemed that the animal would bear watching but on which we were unable to make a positive diagnosis. Every investigator discovers this dilemma because all scorbutic animals become "nervous" or "touchy" while all nervous ones may not have scurvy. The clinical information as listed refers to a determination of the worst condition which was reached at any time during the course of an experiment. Those with no scurvy were obviously free the entire period. The tables do not enumerate re-

lapses or remissions. Although for the most part the scorbutic state is dynamic, some stationary cases were observed.

The method and value of scoring has been previously mentioned. Since a record of pathological findings is distinct from a pathological diagnosis, we have recorded rigorously those conditions which were present even though we were of the opinion that they were not always primarily and in strict fairness to be attributed to the experimental treatment. The latter is indicated in the column under "remarks." Old hemorrhages present quite a problem, as hemosiderin may remain for a long time *in situ* and confuse the unwary, though due to pre-experimental circumstances. Also the post-mortem scores may be exaggerated in some cases, owing to the fact that the hemorrhage *per rhexis* is of traumatic origin (24), while the tendency to have a hemorrhage, is undoubtedly scorbutic. We have ample evidence from other experience that an animal on the scorbutic threshold, presumably with no lesions present which the naked eye would discern, can have a severe muscular hemorrhage as a result of exertion, and give a higher score than his so-called peers or equals. We have used ether in terminating experiments, and thereby avoided the possibility, encountered by some others, of chloroform producing hemorrhages.

The peculiarities of this series of experiments are found chiefly in the experiments in which the larger doses of milk were fed to animals not surviving the full experimental period. One animal receiving 50 cc. of raw milk had definitely scorbutic ribs. One animal receiving 62.5 cc. milk boiled in aluminum had an attack of respiratory disease and pulmonary abscesses containing greenish pus. We suspected arthritis, even ante-mortem, and the wrists were acutely swollen, being much larger than any scorbutic wrists which we had seen up to that time. There was no hemorrhage or congestion about these joints upon gross post-mortem examination and no evidence of scurvy was found elsewhere. Two animals in the 50 cc. group had traces of old shin hemorrhages which we believe were acquired before or perhaps very early in the experiment as result of pre-experimental circumstances. Likewise two animals, one receiving 62.5 cc. of milk boiled in glass and another 50 cc., presented conditions which we assigned to other than direct experimental causes. The effect of these, if one is to interpret them for the worst, is to push the certain protective dose higher into dosages which it is not only impossible but impractical to feed. In view of what we have said, as well as the result of our examination of the quality of commercial animals which we used, and the high incidence of alimentary disturbances in animals in the high dosage groups,

we have disregarded these ocessional irregularities, presenting them here only for the sake of completeness.

There are, nevertheless, enough data to allow us to draw conclusions after the complete rejection of all animals dying during the course of the experiment, in which case the number of unusual results is considerably decreased. The general trends take precedence over too weighty an influence of one experiment on account of the paucity of data in individual groups (even though the total animals used are large in number). A tendency exhibited by, or conclusion drawn from, one group is moreover expected to be strengthened by that from other groups.

Twenty cubic centimeters of raw milk and 25 cc. of each of the boiled milks are practically on a par and 40 cc. of the raw milk and 50 cc. of each of the boiled milks are also about on a par. In the former case practically all animals were afflicted with certain scurvy, either diagnosed by clinical observation or by post-mortem examination or both, whereas in the latter there was a general freedom from scurvy both clinically and pathologically. Intermediate groups bring out intermediate effects. These data, therefore, support the conclusion that boiling of milk, as in our experiments, reduced the concentration of the antiscorbutic potency to 80 per cent of its original value, and that there has accordingly been a 20 per cent loss. They also show that there was no detectable difference between boiling in the two types of vessels. Since glass may be taken for the present as an example of an inert standard, it follows that aluminum is equally good, and that the destruction of that amount of vitamin which did occur, is referable to the boiling process alone.

These data conform to the existence of a range of apparent protective doses, in contradistinction to a precise and one-level protective dose, unless such level be an arbitrary or a statistical one. This has been the experience of other investigators(10). It is also the point of view of Hess (4) when he says that it takes a pint of milk a day to protect an infant, in that he refers to the elimination of practically all cases and not to an incidence of 50 per cent. Moreover, such an experience is well known in pharmacological and toxicological studies, (26), and it would seem important for future investigators to bear this in mind in formulating their schemes of attack when assaying vitamin C.

The individual gains in weight and food intake are given in Tables VIII, IX, and X. No animals are included which showed any pathology other than scurvy, even though the pulmonary lesions were entirely localized or of an incipient type. The reason for this exclusion is that we have found by extensive study that this condition is frequently asso-

TABLE V
SUMMARY OF CLINICAL HISTORIES AND POST-MORTEM EXAMINATION OF GUINEA PIGS FED RAW MILK

Group Dose	Duration	Guinea Pig No.	Degree of Clinical Scurvy: Free	Doubtful	Slight	Moderate	Severe	Autopsy: Score	Remarks
cc.	days								
50	90	233	x					0	
	90	236	x					0	
	90	285	x					0	
	90	358		x				0	
	90	420		x				0	
	90	459	x					2	Ribs over grown. Cause?
	Av. 90 days			6	0	0	0	0.33	
	66	243	x					0	
	Av. less than 90 days			1	0	0	0		
	Grand Total			7	0	0	0	0.33	
40	90	223	x					0	
	90	227	x					0	
	90	231	x					0	
	90	247	x					0	
	90	269	x					0	
	90	230	x					0	
	90	385	x					0	
	Av. 90 day and Grand Total			7	0	0	0	0	
32	90	249		x				2	
	90	263				x		2	
	90	282	x					0	
	90	284		x				1	
	90	306	x					0	
	90	402		x				0	
	90	504			x			0?	
	Av. 90 day			5	1	1		0.7	
	80	328		x				1.5	
	85	311			x			1.5	
	Average less than 90 days			1	1			1.5	
	Grand Total			6	2	1	0	0.9	

TABLE V—*continued*

			Degree of Clinical Scurvy					
25	90	391	x				2	
	90	396			x		1	
	90	416		x			1.5	
	90	445		x			0.5	
	90	450	x				3.	Recent haem.
	90	452	x				1.5	violent injury.
	Av. 90 day and Grand Total		5		1	1	1.6	
20	90	509		x			5	
	90	511				x	1	
	90	514				x	7	
	90	517				x	6	
	90	523			x		6	
	90	525		x			0?	
	90	527			x		3.5	
	Av. 90 days		2		1	2	4.1	
	80	512			x		11.5	
	87	515		x			3	
	Average less than 90 days		3		2	0	0	7.25
	Grand Total		3		2	2	4.8	

ciated with poor growth and appetite and with the shortening of life of animals receiving no vitamin C in their diets (negative controls). Although in some animals this does not appear to be the case, we have, nevertheless, adopted the policy of eliminating such experiments from immediate consideration.

By comparison with those animals recorded in Table II it is seen that the animals fed milk by pipette grew rather poorly. We are inclined to attribute this to lack of appetite in some cases, since the growth per gram of food consumed was sometimes as great as in those animals fed the basal control. It should be noted, however, that these latter animals received spinach which furnished additional vitamins, whereas the experimental animals received only milk. We do not know what the effect of heating the milk powder used in our diet was on other or unknown vitamins. As the data stand, there are insufficient experiments in each group to strike a significant average growth, considering the possible individual variation which is within normal limits (see Nos. 334 and 421, Table II).

TABLE VI
SUMMARY OF CLINICAL HISTORIES AND POST-MORTEM EXAMINATION OF ALL GUINEA PIGS FED MILK BOILED IN ALUMINUM

Group Dose	Duration	Guinea Pig No.	Degree of Clinical Scurvy: Free	Doubtful	Slight	Moderate	Severe	Score	Autopsy: Remarks
cc. 62.5	days 90	235	x					0	
	90	303	x					0	
	Av. 90 days		2		0	0	0	0	Died suddenly. Lung Abscess.
	81	360	No diagnosis made			arthritis suspected		3?	No certain scurvy macroscopically. Foci of infection in wrist found histologically.
	63	273	x					0	
	54	248	x					0	
	63	364	x					0	
	Av. less than 90 days		4		0	0	0	0.75	
	Grand Total		6		0	0	0	0.5	
50	90	220	x					0	
	90	262		x				?1	Shin: Old healed haem. prob. pre-experimental
	90	276	x					0	
	90	382	x					0	
	90	390	x					0	
	90	403	x					0	
	90	408		x				0	
	90	473	x					0.5	Knee: Trace of old haem. prob. pre-experimental.
	Av. 90 days and Grand Total		8		0	0	0	0.19	
40	90	255		x				0	
	90	272	x					0	
	90	325	x					0	
	90	330		x				0	
	90	424		x				3	
	90	469		x				1	
	Av. 90 days		6		0	0	0	0.67	
	68	302	x					0	
	Av. less than 90 days		1		0	0	0	0	
	Grand Total		7		0	0	0	0.6	

TABLE VI—*continued*

			Degree of Clinical Scurvy				
32	90	386	x			0?	
	90	518		x		0	
	90	567			x	6	
	90	570		x		0	
	Av. 90 days		1	2	1	1.5	
	78	412	x			1	
	57	569		x		1	
	Av. less than 90 days		1	1		1	
	Grand Total		2	3	1	1.3	
25	90	524			x	7	Easy bleeder
	90	532		x		3	
	90	533	x			1	
	90	554		x		1	
	90	568		x		1	
	Av. 90 days		1	3	1	2.6	
	51	520		x		0	
	81	522			x	7	
	48	530		x		0.7	
	Av. less than 90 days		0	2		2.3	
	Grand Total		1	5	1 1	2.5	

Cavenaugh, Dutcher and Hall (8) used growth curves, but their data are more numerous per group than ours. Kenny (23) used growth curves in tomato juice experiments. MacLeod(12) omitted consideration of growth in milk experiments similar to ours.

The data obtained from the experiments in which 25 cc. and 32 cc. of milk boiled in glass were fed are unique in that they show these animals tended to exceed some other animals in growth or were about as good as the best. However, there is here shown a tendency for a greater degree of scurvy to appear at autopsy. This might be expected under these circumstances, and definitely indicates that more data than we have are necessary before relying upon growth curves.

TABLE VII

SUMMARY OF CLINICAL HISTORIES AND POST-MORTEM EXAMINATION OF ALL GUINEA PIGS FED MILK BOILED IN GLASS

Group Dose	Duration	Guinea Pig #	Degree of Clinical Scurvy: Free	Doubtful	Slight	Moderate	Severe	Autopsy: Score	Remarks
cc. 62.5	days 90	335	x					0	Prob. not an experimental affair.
	90	480	x					½?	
	90	483	x					0	
	Av. 90 days		3	0	0	0	0	0.16	
	52	349	x						
	Av. less than 90 days		1	0	0	0	0	0	
	Grand Total		4		0	0	0	0.1	
50	90	250	x					0	
	90	264		x				0	
	90	305	x					0	
	90	345	x					0	
	90	427	x					0	
	Av. 90 days		5		0	0	0	0	
	81	455	x					0	May partially or all have been pre-experimental
	45	379	x					3?	
	52	359	x					0	
	45	381	x					0	
	Av. less than 90 days		4		0	0	0	0.75	
	Grand Total		9		0	0	0	0.33	
40	90	281	x					0	
	90	291		x				2	
	90	371	x					0	
	90	388			x			1?	
	Av. 90 days		3		1	0	0	.75	

TABLE VII—*continued*

			Degree of Clinical Scurvy					
40	53	324			x		2	
	66	369		x			2	
	51	368	x				0	
	70	307	x				0	
	Av. less than 90 days		3		1	0 0	1	
	Grand Total		6		2	0 0	0.9	
32	90	439				x	5.5	
	90	400				x	5.0	
	90	475		x			1.0	
	90	498		x			0.0	
	90	537			x		1.5	
	Av. 90 days		2		1	2 0	2.6	
	66	497			x		1	
	53	573		x			0	
	52	448				x	5.5	
	Av. less than 90 days		1		1	1 0	2.2	
	Grand Total		3		2	3 0	2.4	
25	90	543		x			1	
	90	544		x			6.6	Recent Haemorrhage.
	90	550	x				3.5	
	90	558	x				1	
	Av. 90 days		4				3	
	72	564				x	10	
	60	542		x			0?	
	Av. less than 90 days		1				5	
	Grand Total		5		0	1 0	3.7	

In the last column of Tables VIII, IX, and X, the clinical condition of each animal is entered. No pathological condition other than scurvy was to be found in the experiments recorded in these tables. These data lead to the same conclusion that we drew from consideration of all animals, (Tables V, VI, and VII) namely, that about 20 per cent of the vitamin C was destroyed in boiling.

TABLE VIII
90 DAY EXPERIMENT ON RAW MILK

Group Dose	Animal No.	Weight		Gain	Food Consumed			Gain per gm. Food	Scurvy Score	Clinical[1] Status
		Start	End		Dry Food	Milk (dry basis)	Sum			
cc.		gms.	gms.	gms.	gms.	gms.	gms.			
50	420	317	467	150	826	596	1422	.105	0	Doubtful
	236	304	456	152	967	568	1535	.099	0	0
40	230	338	523	185	1257	506	1763	.105	0	0
	247	292	494	202	1109	439	1548	.130	0	0
	269	364	630	266	1373	586	1959	.136	0	0
	231	315	480	165	959	473	1432	.115	0	0
32	282	334	481	147	1120	399	1519	.097	0	0
	306	371	468	97	1096	444	1540	.063	0	0
	504	307	500	193	1316	371	1687	.114	0	0
	284	388	412	24	815	467	1282	.019	1	Doubtful
25	391	331	491	160	1460	309	1769	.090	2	0
	396	344	378	34	1016	321	1337	.025	1	+
	416	297	476	179	1189	281	1470	.122	1.5	Doubtful
	445	283	452	169	1175	264	1439	.118	0.5	Doubtful
	450	318	478	160	1148	273	1421	.113	3	0
	452	293	450	157	1167	276	1443	.109	1.5	0
20	509	306	408	102	1194	231	1425	.072	4	Doubtful
	511	307	485	178	1624	231	1855	.096	1	++
	514	324	272	—52	1169	242	1411	—	7	+++
	517	322	434	112	1536	242	1778	.063	5	+++
	523	345	473	128	1616	259	1875	.068	4	++
	525	321	396	75	1082	242	1324	.057	0	Doubtful

[1] "Doubtful" means not certainly diagnosable, and plus marks the degree of scurvy.

SUMMARY AND GENERAL DISCUSSION

An experiment has been reported on the destruction of Vitamin C in milk by boiling, based upon the development of experimental scurvy in guinea pigs. The data do not permit the mathematical expression of results with a precision of a few per cent. They do, however, indicate that there was no detectable difference in the destruction of vitamin C between boiling milk in aluminum and boiling in glass. The loss of vitamin is placed at about 20 per cent. If glass may be taken as an inert standard, it is certain that no detectable catalysis by the aluminum is evident; rather, as was to be expected, the effect is referable to the cooking *per se*.

The data are also of interest in that our winter milk was approximately if not actually of the same potency as the best summer milk obtained from cows on pasture(9). If a pint of ordinary city milk is required to insure the protection of infants from scurvy, and if such milk protects guinea pigs when fed at the 80 to 100 cc. level (4), it can be said that the milk used by us was at least twice as potent, even in the boiled state, as ordinary city delivered milk in New York City. We would estimate that

TABLE IX
90 DAY EXPERIMENT ON MILK BOILED IN ALUMINUM

Group Dose	Animal No.	Weight		Gain	Food Consumed			Gain per gm. Food	Scurvy Score	Clinical[1] Status
		Start	End		Dry Food	Milk (dry basis)	Sum			
cc.		gms.	gms.	gms.	gms.	gms.	gms.			
62.5	235	296	553	257	799	698	1497	.172	0	0
50	276	290	441	151	839	546	1385	.109	0	0
	382	357	517	160	996	669	1665	.096	0	0
	473	306	414	108	954	574	1528	.071	0.5	0
	390	313	458	145	869	585	1454	.100	0	0
40	255	345	561	216	1317	518	1835	.118	0	Doubtful
	325	347	525	178	1040	523	1563	.114	0	0
	469	290	440	150	820	438	1259	.119	1	Doubtful
	272	323	441	118	1004	484	1488	.079	0	0
32	570	316	405	149	973	377	1350	.110	0	+
	518	364	554	190	1240	439	1679	.113	0	+
25	554	329	390	61	942	309	1251	.048	1	++
	568	344	449	105	1094	321	1415	.074	1	+
	533	387	518	131	1306	360	1666	.079	1	Doubtful

[1] "Doubtful" means not certainly diagnosable, and plus marks the degree of scurvy.

200 cc. daily of our raw milk less than 12 hours old would probably suffice for the infant, but of course we would recommend more for the sake of safety. Dutcher(27) has had the general experience that 30 cc. of milk will protect 250 gram guinea pigs, and his observation is to be accounted for by the fact that poorly produced and handled milk is an unknown quantity at Agricultural Experiment Stations and Colleges in the United States.

The data are of importance in that the destruction was of the order of only 20 per cent, which is in complete conformity with the historical

fact that boiled milk has adequately served in the past as the sole or chief source of antiscorbutic vitamin for some artificially fed infants. However, this historical fact applies to less strenuous circumstances than operate in a modern city; and therefore, by elimination, the problem is to obtain potent milk to begin with.

The data are also of considerable interest in emphasizing the necessity of some rigorous definition of the term "protective dose" at least for the

TABLE X

90 DAY EXPERIMENT ON MILK BOILED IN GLASS

Group Dose	Animal No.	Weight		Gain	Food Consumed			Gain per gm. Food	Scurvy Score	Clinical[1] Status
		Start	End		Dry Food	Milk (dry basis)	Sum			
cc.		gms.	gms.	gms.	gms.	gms.	gms.			
62.5	335	288	470	182	540	675	1215	.150	0	0
	480	316	516	200	631	743	1374	.145	½?	0
	483	292	499	207	708	687	1395	.148	0	0
50	250	285	480	195	962	534	1496	.130	0	0
	305	326	527	201	874	613	1487	.135	0	0
	427	344	552	208	1152	647	1799	.116	0	0
40	291	274	504	230	1227	411	1638	.140	2	Doubtful
32	439	265	488	223	1493	321	1814	.123	4.5	++
	400	335	493	158	1490	399	1889	.084	5.0	++
	475	297	500	203	1311	354	1665	.122	1.5	0
	537	400	665	265	1354	478	1832	.145	1.5	Doubtful
25	544	367	634	267	1451	343	1894	.141	6.5	Doubtful
	550	337	659	222	1410	315	1726	.129	3.5	0

[1] "Doubtful" means not certainly diagnosable, and plus marks the degree of scurvy.

basis of assaying, even if not for computing optimum allowances for the animal. The data fall in line with the experience of pharmacologists who recognize a latitude between the least dose producing a given degree of an effect and a dose producing it in practically all cases(26). This therefore brings into an assay of this type a statistical aspect, regardless of whether arithmetical computations employed by statisticians are or are not performed. It might be imagined that discrepancies between the value of equal-size doses would occur in an animal from day to day, much as with single doses of a medicament given to different animals at the same time. It would appear plausible under these circumstances that

unequal effects from equal doses of vitamin repeated daily would reach an average in the long run, but the theory unfortunately does not work out in practice, as evidenced by remissions of disease in the same animal, or by a partial incidence of disease in animals at a single dose level. When this is realized, it is only one step further to conclude that the agreement in a small amount of data is certainly not indicative of high accuracy, even in assays involving repeated daily feeding.

The growth curves and food intake data were of no help in drawing our conclusions. This is not to be interpreted as a general prediction for the future.

Potent milk is absolutely essential to the success of an experiment of this sort. While the pipette feeding of milk to guinea pigs does not appear to be the best biological practice, and apparently influences appetite adversely, we believe that it is superior to cup feeding, which introduces spillage, aging, cream consumption, and varying daily doses.

Conclusions

The amount of antiscorbutic vitamin destroyed by lightly boiling three quarts of milk for 5 minutes in a glass beaker or in an aluminum stew pan was, within the limits of accuracy of experiments of this kind, found to be approximately 20 per cent.

The quality of the winter milk used and obtained from ensilage-fed cows was better than any heretofore reported upon and almost, if not actually, as potent as the best summer milk obtained from cows on pasturage. This points to a forgotten but possible high potential value of milk as a carrier of vitamin C.

The amount of aluminum contributed to Pittsburgh tap water and fresh milk upon boiling in an aluminum vessel is practically nil, being 0.1 to 0.4 parts per million.

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A STUDY OF THE VITAMIN B COMPLEX* OF RED KIDNEY BEANS AND POLISHED RICE†

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IT DOES not seem necessary to review in this paper the literature pertaining to vitamin B and the researches which have shown its multiple nature, since this has been summarized and discussed in the recent literature pertaining to the complex nature of vitamin B, especially in an editorial review by Sherman (1928) and in a dissertation by Chase (1928).

Sherman and Axtmayer (1927) devised a method by means of which they determined which of the two now recognized factors of the vitamin B complex, *viz.*, the antineuritic or vitamin B and the pellagra-preventive or vitamin G, is the limiting factor in whole wheat flour and in milk powder. In view of the fact that highly polished rice and red kidney beans, served either separately or together, is a dish which is consumed daily by the people of Porto Rico, at the sacrifice of other more nutritive foodstuffs, it was decided to apply this method to determine whether vitamin B(B_1) or vitamin G(B_2) is the limiting factor of the "vitamin B complex" of this mixture. It has been shown that foodstuffs may be richer in one of the factors than in the other, hence a reinvestigation and reinterpretation of the previously recorded "vitamin B values" of foodstuffs becomes a necessity in order to know whether there exists any supplementary relationship between any two insofar as the vitamin B complex content is concerned. The relative richness of any one factor should also be determined because the concentration of these two factors is not the same in all foods, and a foodstuff rich in one may be relatively poor in the other.

Experimental

The experiments were conducted according to the methods of Sherman and Spohn (1923), and Sherman and Axtmayer (1927) for the quantitative determination of vitamin B values of foods. These methods involve placing healthy young rats when 28 to 29 days old upon a vitamin B-free diet which is not only adequate but approximately optimal (for growth of rats) in all other respects. The basal diet consists of purified casein,

* The term "vitamin B complex" is used in this paper to signify the combination of the thermo-labile factor, vitamin B (B_1) and the thermo-stable factor, vitamin G (B_2).

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18; Osborne and Mendel (1919) salt mixture, 4; butterfat, 8; cod-liver oil, 2; corn starch, 68 per cent. The corn starch was such as has been found by Osborne, Wakeman and Ferry (1919) to be free from vitamin B.

The foods tested in the experiments described in this paper were polished rice, autoclaved yeast, and red kidney beans. The rice and beans, which were of the same kinds as used in the Porto Rican homes, were purchased from a local grocer and were freed from dust and extraneous matter. They were then ground separately, the rice in a mortar and the beans in a meat grinder. Dried baker's yeast was placed in uncovered Petri dishes to a depth of 15 mm. and heated in an autoclave at 15 pounds steam pressure for 150 minutes; then, after cooling and drying in the air, it was ground to a powder in a mortar.

These test foods were fed separately or in combination as sole sources of vitamin B to the standard test animals on the basal vitamin B-free diet.

The multiple nature of vitamin B having been established, it was deemed advisable to determine the relative richness of the factors of the vitamin B complex in polished rice and red kidney beans by the same method which is based on the fact that if better growth is induced by the feeding of a mixture of foods as the source of vitamin B complex than is obtained when double the amount of each is fed separately, then, all other conditions having been properly controlled, the conclusion is justified that the better growth is due to a supplementation of one of the factors of the vitamin B complex by the other.

Non-supplementary Relation between Polished Rice and Red Kidney Beans

After some preliminary work a series of experiments was performed in which the experimental animals (rats) received as the sole source of vitamins B and G the following daily (six days per week) portions of the test foods: 0.8 grams of ground red kidney beans; or 0.8 grams of ground polished rice; or 0.8 grams of autoclaved yeast; or a mixture of 0.4 grams each of the rice and red kidney beans; or a mixture of 0.4 grams each of the rice and autoclaved yeast; or a mixture of 0.4 grams each of the beans and autoclaved yeast; with the negative control experiments in which animals received the vitamin B-free basal diet. The 0.8 grams level of feeding the supplements was chosen in order that the results obtained be directly comparable with those obtained by Sherman and Axtmayer (1927).

The average weight curves of those animals receiving daily portions of 0.4 grams each of the red kidney beans and the autoclaved yeast;

0.4 grams each of the polished rice and the red kidney beans; 0.8 grams of the autoclaved yeast alone; 0.8 grams of the red kidney beans alone, and the negative controls, are shown in Fig. 1.

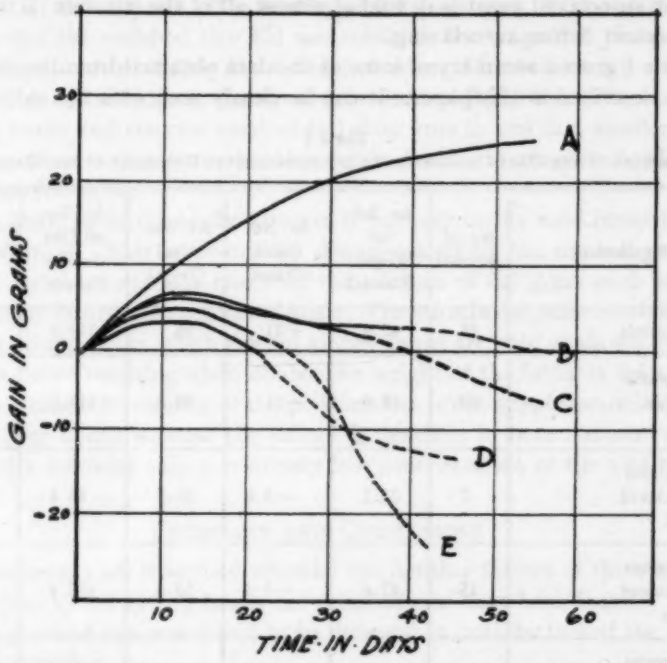

FIG. 1.—Average weight curves of directly comparable groups of experimental animals (rats of initial age of 28 to 29 days) receiving a basal diet devoid of the vitamin B complex but good in all other respects, and in addition: Curve A, 0.4 grams of red kidney beans plus 0.4 grams of autoclaved yeast (daily except Sundays); Curve B, 0.8 grams of red kidney beans (daily except Sundays); Curve C, 0.4 grams of polished rice and 0.4 grams of red kidney beans (daily except Sundays); Curve D, no other food,—these are the negative controls; Curve E, 0.8 grams of autoclaved yeast (daily except Sundays). The average weight curve for each group is represented by a solid line to the point at which the first death occurred among the experimental animals, and from that point onward by a broken line representing the average weight of the surviving experimental animals of the group until the end of the 8 weeks experimental period.

Curve A shows marked supplementation while Curves C and E show deficiencies of vitamin B (B_1) of the vitamin complex.

A comparison of these growth curves will show at once that there was no supplementary relationship when the mixture of red kidney beans and polished rice was fed, while, on the other hand, a marked supplementary relationship and not merely an additive effect was shown when the mixture of red kidney beans and the autoclaved yeast was fed. Evi-

dently the vitamin B complex deficiencies of the beans and the rice are not supplemented by each other.

The results obtained in these experiments confirm those of other workers in that autoclaved yeast is devoid of almost all of the vitamin B which was present before autoclaving.

Table I gives a summary of some of the data obtained from the experiments described in this paper. It can be clearly seen with the aid of the

TABLE I

SUMMARY OF THE DATA OBTAINED FROM THE EXPERIMENTS DESCRIBED IN THIS PAPER

Supplement	No. of Animals	Av. Initial Weight, (Grams)	Av. Net Gains (Grams)	Av. Survival (Days)	Av. Basal Diet Intake (Grams)	Per cent Polyneuritic.
Controls	15	42.9	—11	26	119.9	80
0.8 grams polished rice	20	40.6	—11	50.3	118.8	100
0.8 grams autoclaved yeast	7	35.7	—3.6	26.8	66.4	57
0.8 grams red kidney beans	15	47.6	—5.3	53.4	182.4	40
0.4 grams polished rice plus 0.4 grams autoclaved yeast	10	53.0	—6.4	46.8	188.5	100
0.4 grams red kidney beans plus 0.4 grams autoclaved yeast	12	51.8	25.0	56	263	0
0.4 grams red kidney beans plus 0.4 grams polished rice	23	53.3	—3.5	53.8	174.2	48

growth curves and this table that 0.8 gram of polished rice furnishes very little vitamin B(B_1) as shown by the number of rats developing polyneuritis, while the 0.8 gram of red kidney beans furnishes a significant amount of this vitamin. The feeding of 0.4 gram each of the red kidney beans and the polished rice did not result in better growth than when the 0.8 gram of red kidney beans was fed alone. This validates the statement that the red kidney beans contain vitamin B(B_1) since the mixture of the beans and the rice resulted in better growth and in a smaller percentage of cases of polyneuritis than when double the amount of polished rice was fed alone.

The limiting factor of the vitamin B complex in the red kidney beans is vitamin G. This is beautifully demonstrated by the marked growth shown by those animals receiving the mixture of 0.4 gram each of the red kidney beans and autoclaved yeast. The autoclaved yeast is relatively rich in vitamin G in which the red kidney beans are poor as shown by the growth curve resulting when double the weight of the latter is fed alone.

The vitamin B complex of the polished rice is not supplemented by the red kidney beans because the former is deficient in both factors while the latter contains only a relatively low concentration of the vitamin G factor.

Summary and Conclusions

Experiments are described whereby the limiting factors of the vitamin B complex in red kidney beans and polished rice have been found.

The polished rice was found to be deficient in both factors of the vitamin B complex.

Vitamin G was found to be the limiting factor of the vitamin B complex of red kidney beans which are therefore relatively richer in vitamin B(B_1) than in vitamin G(B_2).

The vitamin B deficiency of the polished rice which was used in these experiments is not made good by feeding the rice with red kidney beans, for the former was deficient in both factors while the latter was poor in vitamin G.

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THE GASEOUS EXCHANGE OF THE HUMAN SUBJECT I. AS AFFECTED BY INGESTION OF WATER AT 37°C

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THE purpose of the experiments reported here was to study the gaseous exchange, the calculated heat production and the carbohydrate metabolized in periods following the ingestion of small quantities of water (50 to 500 cc.) at 37°C. Experiments (to be reported in papers following this one) were made to determine the influence of the ingestion of small amounts of levulose and dextrose. Water at 37°C was given with these sugars with the idea, based upon findings in the previous literature, that the water alone would have little or no effect upon the total metabolism either qualitatively or quantitatively. In order to be certain whether the ingestion of water at body temperature would or would not have an effect upon the total metabolism, the experiments in this paper were made with water alone. In these experiments, as in the sugar experiments, the same routine was observed of basal measurements, a rest period, and measurements extending over several hours following the ingestion of the water.

Previous investigations. The effect of the ingestion of water has been studied by Laschtschenko (1898), who observed no change in elimination of carbon dioxide when the temperature of the water was 37°C. Speck (1892) did not report the temperature of the water used in his study. Benedict and Carpenter (1918, p. 140) reported a number of experiments but in none was the water at 37°C. Pollitzer and Stolz (1925) gave lemonade with saccharin, but no temperatures were recorded. Cannon, Querido, Britton and Bright (1927) reported a series of experiments in which warm water was given, but in only one of the experiments was the water nearly 37°C. Their subject A.Q. was given 750 cc. of water at 34.5°C and the maximum increase in heat production was 5.4 per cent 11 minutes after the water was ingested. Lublin (1928) found an increase in the metabolism of from 3 to 8 per cent above the basal value within 2 hours after the ingestion of 800 to 1000 cc. of water (temperature not recorded). Grollman (1929) found 5 to 10 per cent rise in the oxygen consumption within a half hour after the ingestion of 1000 and 1200 cc. of water at 38°.

Methods of Investigation

The respiratory exchange was determined by the gasometer method in nearly half of the experiments in this paper. The apparatus used were mouthpiece, two Tissot valves, and two Tissot gasometers of 100 liters capacity which were connected in such a way that determinations could be made for a continuous series of periods. Analyses of the expired air were made in duplicate with the Haldane portable apparatus, and usually by two analysts with two of these gas analysis apparatus. Outdoor air was analyzed each morning with each apparatus before it was used for expired air. The basis for repetition of analyses of expired air was a disagreement of more than 0.02 in the respiratory quotients obtained in the duplicate analyses.

The respiratory exchange was also measured by means of the Benedict Universal respiration apparatus, which was used in the same manner as with the chamber respiration apparatus, that is, with duplicate sets of absorbers for carbon dioxide. The change from one period to another was made by shifting the valves controlling the passage of the air through the sets of absorbers. At the end of each period the height at which the spirometer bell stood was noted and in the computations a correction was made for the error due to the compression of the gas in the absorber system and for the change in the position of the spirometer bell during the period. A graphic record of the breathing of the subject, as well as of the oxygen absorption in short periods of time, was obtained by using a kymograph with continuous paper. The tightness of the closed circuit was tested during each period by placing a weight (Benedict 1921) upon the spirometer bell for a certain length of time and noting the slope of the record of the breathing as compared with that obtained when no weight was used.

In both groups of experiments with gasometer and with Universal apparatus, absence of sleep was controlled by applying the method previously described (Benedict and Carpenter, 1918, p. 354; Carpenter, 1925) in which a signal magnet providing a stimulus operated in unison with another signal magnet recording upon the kymograph. The subject was requested to respond to the stimulus by pressing a button which controlled the recording of a third signal magnet.

The general procedure in an experiment was as follows: The subject, J. C., whose age was 43 yrs., height, 167 cms., nude weight, 66 kg., came to the Laboratory without breakfast and lay resting upon a comfortable couch for at least one-half hour. The mouth-piece was then inserted, the nose-clip put on, and measurements of the metabolism were begun

as soon as possible. During the experiment there was usually a light covering over the subject in addition to his clothing and the temperature of the room was not far from 20°C. Six continuous periods, each of 10 to 15 minutes' duration, were first run in order to obtain the basal value for the day. A rest period, varying in length in the different experiments between 15 and 30 minutes, then followed after which the water was given, the quantity having previously been measured and warmed to 37°C. Within one to six minutes after the subject finished drinking the water, the mouthpiece was inserted and observations of the metabolism were resumed in 10 to 15 minute periods which were continuous for 1½ to 2½ hours. A second rest period of from 15 to 30 minutes was taken. The experiment was then concluded with a series of periods, which in most of the experiments was continuous for 1 to 2 hours.

Results of Experiments with Water

The water was given by mouth in volumes that varied from 50 to 500 cc. It was taken as quickly as possible, usually by sucking through a small rubber tube. Of the larger quantities (100 cc. or over), all but 25 cc. was first given, and then the 25 cc. for rinsing, in order to imitate as closely as possible the procedure used in the series with dextrose and levulose. The results of the experiments are given in the following tables, the values representing averages for the respiratory quotient, the carbohydrate metabolized, and the heat produced per half hour in the basal periods and in the successive half hours following the ingestion of water.

Respiratory Quotients.

The respiratory quotient is the most important factor measured in this study and in the two succeeding studies, because the object of the experiments with the sugars was to determine the changes in the carbohydrate metabolized when small amounts of either dextrose or levulose were ingested. With the small quantities given it is to be expected that the changes will not be large, and unusual significance therefore has to be attached to slight changes in the respiratory quotient. The breathing appliance used was the mouthpiece. Frequently with untrained subjects this results in distorted breathing, so that the respiratory quotient obtained may not be the true metabolic index. The subject of these experiments was one who had served in hundreds, if not thousands, of periods of observation, in which the mouthpiece was used either with a closed circuit or an open circuit respiration apparatus. He was, therefore, as thoroughly trained to the use of this appliance as one could expect;

TABLE I
SUMMARY OF RESPIRATORY QUOTIENTS AFTER INGESTION OF WATER

Date and apparatus[1]	Amount of water	Basal[2]	Half hours after ingestion of water								
			1	2	3	4	5	6	7	8	9
1926	cc.										
Jan. 20 U	50	0.88	0.88	0.84	0.83	0.86	0.85	0.85	0.84	0.83	0.83
May 17 U	50	.91	.88	.87	.86	.87	.86	.87	.84	.86[3]	
Feb. 15 G	50	.81	.80	.81	.80	.80	.79	.79	.79	.79	.78
May 28 G	50	.85	.85	.83	.82	.85	.85	.84	.82	.82	
	Average	.86	.85	.84	.83	.85	.84	.84	.82	.83	.81
Jan. 15 U	100	.88	.89	.86	.86	.84	.85	.84	.83	.86	.84
Feb. 8 U	100	.85	.88	.85	.83	.84	.84	.86	.84	.82	.83
Feb. 10 G	100	.85	.85	.86	.87	.86	.85	.84	.83	.85	.83
Apr. 28 G	100	.82	.83	.84	.84	.84	.81	.81	.81	.81	
	Average	.85	.86	.85	.85	.85	.84	.84	.83	.84	.83
Jan. 11 U	150	.89	.86	.87	.88	.85	.88	.84	.82	.85	.81[3]
Apr. 30 U	150	.87	.87	.84	.85	.87	.85	.84	.82	.86[3]	
Dec. 14[4] G	150	.83	.82	.84	.83	.83	.83	.82	.82	.81	.81
May 24 G	150	.82	.81	.80	.81	.81	.81	.83	.81	.81	
	Average	.85	.84	.84	.84	.84	.84	.83	.82	.83	.81
Jan. 4 U	200	.90	.87	.86	.83	.84	.84	.84	.84	.80	.82
May 12 U	200	.87	.87	.88	.87	.86	.86	.84	.85[3]		
Nov. 23[4] G	200	.86	.86	.84	.85	.84	.85	.83	.84	.84	.84
Dec. 1[4] G	200	.80	.82	.84	.83	.81	.80	.80	.80	.79	.79
	Average	.86	.86	.86	.85	.84	.84	.83	.83	.81	.82
Dec. 30[4] U	250	.80	.82	.80	.78	.80	.82	.81	.81	.80	.81[3]
Mar. 8 U	250	.83	.84	.84	.84	.84	.84	.81	.82[3]		
Nov. 16[4] G	250[5]	.81	.81	.84	.77	.80	.81	.82	.80	.80	.81
Dec. 23[4] G	250	.82	.82	.82	.78	.81	.80	.81	.81	.81	.80
May 19 G	250	.81	.81	.82	.82	.81	.81	.81	.80	.81	
Sept. 16 G	250	.87	.84	.84	.85	.84	.84	.84	.82	.82[3]	
	Average[6]	.83	.83	.82	.81	.82	.82	.82	.81	.81	
Dec. 17 G	500	.81	.82	.83	.83	.83	.78	.80	.81[3]		

[1] U = Universal apparatus; G = Gasometer apparatus.
[2] Average respiratory quotient for five 10-minute periods.
[3] Respiratory quotient for less than a half hour (15 to 28 minutes).
[4] 1925.
[5] Temperature of the water on Nov. 16, 1925 was 16.2°C.
[6] Does not include results of experiment on Nov. 16, 1925.

in fact, he could endure experiments of two hours' duration without interruption. Earlier studies (Carpenter 1915) have shown that the average respiratory quotient obtained with the Universal apparatus agrees with the average obtained with the gasometer apparatus, and later a special study (Hendry, Carpenter and Emmes 1919) showed that another type of closed circuit apparatus gave results agreeing in the main with those obtained with the gasometer apparatus. It is therefore believed that reliance may be placed in the respiratory quotients found.

The respiratory quotients, which represent the total gaseous exchange, are summarized in Table I for the experiments with both the Universal apparatus and the gasometer apparatus.

The determination of the basal metabolism for the day in these experiments differs from the procedure in most of the previous investigations in which short periods have been used. Thus, the average basal figure given in the tables is the result of the measurement of the respiratory exchange in six succeeding periods without interruption, of which the first was rejected because in the majority of instances this differed more from the average value than did any one of the succeeding five. It seemed as though this first period of 10 to 15 minutes was one of adjustment. Since the succeeding five periods were continuous, if one period gave an aberrant quotient, it should be followed by a period in which compensation took place, and it is considered that the average of five succeeding periods measured in this way should come very close to the real basal respiratory quotient. In a similar way the measurement of the respiratory quotient after the interval of rest, whether water was given or sugars were given, was made in an uninterrupted series of periods extending over one and one-half to two hours. The average result for three 10-minute periods in each of the successive hours following the ingestion of water would therefore represent very nearly the true respiratory quotient for a half hour, because opportunity was given for compensation. There were no lapses in these successive measurements except for a short rest period, which occurred about one and one-half hours after the ingestion for the day took place.

As will be seen from Table I, there was a wide variation in the average basal respiratory quotients on the different experimental days, many being high and all of them 0.80 or over. The successive values for periods 2 to 6 on any one day, however, were uniform even when the respiratory quotient was high, as seen in the illustration in Table II, taken from the experiment with the Universal apparatus preceding the ingestion of water on May 17, 1926. An analysis of the basal respiratory quotients

in periods 2 to 6 shows the following: With the Universal apparatus the range of five periods varies from 0.022 on December 30 to 0.077 on January 4; the average range is 0.042. If, however, the best four of the five periods are selected this range is lowered slightly to from 0.054 on January 15 to 0.011 on January 11, May 12, and December 30, and the average range is 0.026. The average range of five periods with the gasometer apparatus is 0.033, and the average range of the selection of the best four is 0.021. The difference between the average quotient with five periods and four periods with the Universal apparatus varies from 0.011 to 0.004, and the average of the average quotients of five periods is 0.868 as compared with

TABLE II
SAMPLE OF PROTOCOLS OF MEASUREMENTS OF BASAL METABOLISM

Period	Time	Duration of period	CO_2 per minute	O_2 per minute	Respiratory quotient
		min.	cc.	cc.	
1	9.02-a.m.	10.33	200	209	0.96
2	9.12- "	11.42	190	204	.93
3	9.23- "	10.78	189	210	.90
4	9.34- "	11.00	192	208	.92
5	9.45- "	10.78	189	208	.91
6	9.56- "	11.45	191	211	.91

0.867 for four periods. With the gasometer apparatus the difference between the average of five periods and the average of four periods is less than 0.010 in all cases, and the average quotient is identical when all the experiments are taken into consideration. In general, then, it does not make any difference whether four periods or five periods are selected for the average basal quotient. In other words, there is a high degree of uniformity in the five successive measurements which are selected for the average basal quotient. Therefore, small differences in the average respiratory quotient from half hour to half hour are of significance. If there was actually a change, for example, in the respiratory quotient over basal of 0.01, and this persisted for several periods, the change is considered to be significant.

METABOLISM OF CARBOHYDRATE

The respiratory quotients were computed from measurements of total carbon dioxide and oxygen, which include the amounts resulting from the metabolism of protein. For the computation of total heat production, and consequently the metabolism of carbohydrate, the respiratory ex-

change was apportioned to the different nutrients, the metabolism of protein being calculated by means of the necessary factors and from the average urinary nitrogen for this series (0.47 gram per hour in the experiments with the Universal apparatus and 0.40 gram per hour in the experiments with the gasometer). The amounts of carbohydrate metabolized as calculated for the basal periods and for the half hours following the ingestion of water are given in Table III.

In the experiments with the smaller quantities of water, 50 and 100 cc., there were usually lower respiratory quotients in the half hours following the ingestion of the water than in the basal periods, the decreases beginning for the most part in the first or near the first half hour. The greater decreases in the quotients occurred after the higher basal quotients. The average variations for the half hours in the two groups (50 cc., −0.03; 100 cc., ±0.01) give some indication of the tendency to small variation or actual decrease in the respiratory quotients following the ingestion of small amounts of water.

Following the ingestion of the smallest amount (50 cc.) of water there was a decreased metabolism of carbohydrate in comparison with basal metabolism in all but two of the half-hour periods, the two exceptions being +0.07 and +0.05 gram in the first and second half hours on January 20 and February 15, respectively. The largest decrease followed high basal carbohydrate accompanying high basal respiratory quotient.

Experiments with 100 cc. of water gave results somewhat different from those obtained with 50 cc. of water, even though there was high basal carbohydrate (respiratory quotient, 0.88) in one of the four experiments, for in all the experiments there was increased metabolism of carbohydrate in the first half hour and two of the experiments showed increases in other half hours. The net changes in 4 hours ranged from +1.0 gram to −4.7 grams.

Among the four experiments with 150 cc. of water, the first two were experiments with high basal respiratory quotients, but in neither of these was there a marked change from the basal value until the sixth and seventh half hours (−0.05 and −0.07) after the ingestion of water, so that in relation to high basal quotients there was a delayed drop in the respiratory quotient after this amount of water as compared with the drop in quotient after the smaller quantities of water. The maximum change in the other two experiments, on December 14 and May 24 with normal basal quotients, was −0.02 and on May 24 there was a practically constant respiratory quotient for about five hours. The ingestion of 150 cc. of water was followed by decreased metabolism of carbohydrate in

TABLE III

SUMMARY OF CARBOHYDRATE METABOLIZED AFTER INGESTION OF WATER (Grams per half hour).

Date and apparatus[1]	Amount of water	Basal[2]	Half hours after ingestion of water								
			1	2	3	4	5	6	7	8	9
1926	cc.										
Jan. 20 U	50	3.76	3.83	2.67	2.48	3.08	2.86	2.92	2.52	2.33	2.59
May 17 U	50	4.55	3.80	3.51	3.29	3.40	3.21	3.53	2.52	3.19[3]	
Feb. 15 G	50	2.25	2.08	2.30	2.05	1.83	1.61	1.59	1.60	1.61	1.61
May 28 G	50	3.12	2.94	2.51	2.49	2.89	3.06	2.65	2.08	2.30	
	Average	3.42	3.16	2.75	2.58	2.80	2.69	2.67	2.18	2.36	2.10
Jan. 15 U	100	3.64	3.99	3.17	3.00	2.67	3.19	2.77	2.51	3.08	2.92
Feb. 8 U	100	3.25	3.92	3.03	2.65	2.75	2.81	3.37	2.84	2.24	2.68
Feb. 10 G	100	3.13	3.39	3.44	3.64	3.35	3.17	2.71	2.50	3.19	2.52
Apr. 28 G	100	2.30	2.76	2.81	2.71	2.73	2.05	2.08	2.10	2.13	
	Average	3.08	3.52	3.11	3.00	2.88	2.81	2.73	2.49	2.66	2.71
Jan. 11 U	150	4.24	3.19	3.45	3.62	3.08	3.64	2.52	2.18	2.93	2.07[3]
Apr. 30 U	150	3.49	3.49	2.75	2.88	3.43	3.05	2.67	2.35	3.00[3]	
Dec. 14[4] G	150	2.95	2.50	2.84	2.59	2.59	2.52	2.32	2.35	1.95	2.16
May 24 G	150	2.54	2.24	1.90	2.16	2.09	2.13	2.65	2.25	2.25	
	Average	3.31	2.86	2.74	2.81	2.80	2.84	2.54	2.28	2.53	2.12
Jan. 4 U	200	4.73	4.19	3.67	2.62	3.06	3.13	2.98	3.09	1.97	2.44
May 12 U	200	3.49	3.62	3.69	3.64	3.19	3.37	2.60	3.21[3]		
Nov. 23[4] G	200	3.27	3.33	2.79	2.99	2.71	3.10	2.49	2.70	2.71	2.73
Dec. 1[4] G	200	2.13	2.68	3.12	2.88	2.36	1.93	2.16	2.19	1.72	1.94
	Average	3.41	3.46	3.32	3.03	2.83	2.88	2.56	2.80	2.13	2.37
Dec. 30[4] U	250	1.93	2.58	1.98	1.33	1.85	2.27	2.38	2.15	1.88	2.08[3]
Mar. 8 U	250	2.54	3.01	2.79	3.01	2.79	2.79	2.18	2.19[3]		
Nov. 16[4] G	250[5]	1.93	2.26	2.82	1.39	1.96	1.94	2.32	1.75	1.92	1.90
Dec. 23[4] G	250	2.08	2.08	2.32	1.27	2.12	1.86	2.09	2.07	2.08	1.72
May 19 G	250	2.21	2.30	2.50	2.46	2.25	2.21	2.25	1.79	2.19	
Sept. 16 G	250	3.70	3.19	3.14	3.11	3.09	2.90	2.93	2.27	2.51[3]	
	Average[6]	2.49	2.63	2.55	2.24	2.42	2.41	2.37	2.09	2.17	
Dec. 17 G	500	1.90	2.43	2.62	2.98	2.78	1.45	1.86	2.13[3]		

[1] U = Universal apparatus; G = Gasometer apparatus.
[2] Calculated to one half hour from five 10-minute periods.
[3] Calculated from results for less than a half hour (15 to 28 minutes).
[4] 1925.
[5] Temperature of the water on Nov. 16, 1925 was 16.2°C.
[6] Does not include results of experiment on Nov. 16, 1925.

practically all half hours, whether the basal carbohydrate was high or comparatively low, the decreases averaging for the four experiments 1.28 to 0.33 grams per half hour and the total decrease in 4 hours ranging from 9.31 (basal carbohydrate, 4.24 grams and respiratory quotient, 0.89) to 2.65 (basal carbohydrate, 2.54 grams and respiratory quotient, 0.82). In only one first half hour was there no change and in a single sixth half hour there was an increase of 0.11 gram.

There were three experiments of the four with 200 cc. of water in which the basal respiratory quotient was high; the quotient in the fourth experiment was normal. One of the high basal quotients was 0.90 on January 4 and from this quotient there was a marked drop to quotients lower by 0.07, 0.10 and 0.08 in the third, eighth and ninth half hours, with an average variation of −0.06. In the other two experiments with high basal quotient, the variations after the ingestion of water were low, averaging −0.01 and −0.02, and in the experiment with the normal basal quotient, there was an increased quotient in the first four half-hour periods. In most of the experiments, then, 200 cc. of water delayed or prevented the fall in respiratory quotient that occurred after the smaller quantities of water. In two of the four experiments with 200 cc. of water, there were increases in carbohydrate metabolized in the first two half hours and in one of these two experiments, the increase continued throughout the first four half hours. There is some indication that, with increase in the amount of water ingested, there is less decrease from the basal carbohydrate metabolized and there is even increase in the metabolism of carbohydrate.

In only one of the six experiments with 250 cc. of water was there a high basal quotient, *i.e.*, 0.87 on September 16, from which quotient the variations in the half hours after the ingestion of water were from −0.02 in the third half hour to −0.05 in the seventh and eighth half hours. The other five experiments, including the experiment on November 16, 1925, with water at 16.2°C, showed very small changes from basal respiratory quotients that were all normal, the maximum variation being −0.04 on two different days. In three of the experments, the quotient was higher (+0.03 in one instance) in the second half hour than the basal quotient and in all five the quotient in the first half hour was either slightly higher than the basal quotient or not changed. In each of these five experiments with basal quotients from 0.80 to 0.83, there was a practically constant respiratory quotient for a period of 5 to 6 hours.

Of the six experiments (including the experiment of Nov. 16, 1925, with water at 16.2°C.) there were five in which there was either little or no

decrease in carbohydrate metabolized or else an increase in the first two and one-half hours. This increase persisted in two instances to three and one-half hours.

The single experiment with 500 cc. of water gave results following a normal basal quotient which are somewhat similar to the results in five of the experiments in the preceding group. There was a slightly increased respiratory quotient in the first four half hours after the ingestion of water. There was an increase in metabolism of carbohydrate in the first four half hours above the basal carbohydrate of 1.9 grams (respiratory quotient 0.81) and a net increase in 3 1/2 hours of 3.0 grams.

In general there was the greatest falling off in the respiratory quotient after the ingestion of water when the basal respiratory quotient was high (the higher the initial quotient the greater the potentiality for a fall) and the larger the quantity of water ingested the less was the decrease in the respiratory quotient in the succeeding half hours.

There was a decrease in the amount of carbohydrate burned in the periods subsequent to the ingestion of 50 cc. of water, whether there was a low or high basal metabolism of carbohydrate. When the quantities of water were 100 cc. or over, there were in some cases increases in carbohydrate above the basal amount and in others there were decreases, the increases being more marked after the higher quantities of water, and to a certain extent independent of the height of the basal carbohydrate and the basal respiratory quotient. Stated more exactly, there was increased metabolism of carbohydrate when the water was 200 cc. or over, both in comparison with the results of ingestion of smaller amounts of water and in comparison with the basal carbohydrate.

Heat Production

The heat production calculated per half hour for the basal condition and for the half hours after the ingestion of varying quantities (50 to 500 cc.) of water at 37° C. is given in Table IV. The average basal value was 31.1 calories, the maximum (on January 4) being 2.5 calories above the average, that is, 8 per cent. The minimum (on January 15) was 29.5 calories, that is, 5 per cent below the average, so that in the 23 experiments with water at 37° C. there was a range of 13 per cent in the basal heat production.

The heat production after 50 cc. of water showed an increase above the basal value in the first half hour in each of the four experiments, but in three experiments it was progressively lowered during the remainder of four hours after the ingestion of water, the net change in 4 hours being

−4.9, −6.6 and −2.1 calories in the respective experiments. In one experiment, the heat production was increased 1.1 calories in the first hour and then remained practically without change during the remainder of the experiment.

The ingestion of 100 cc. of water, with the exception of the experiment on February 10, was followed for the most part by small changes in heat production of which the largest were +0.9 in the fifth half hour on January 15, −0.9 in the fourth half hour on February 8 and +1.0 calorie in the second half hour on April 28. On February 8 the values decreased after the first half hour and the net change in 4 hours from the basal value was −1.8 calories; on April 28 the values were mostly increased, the total increase being 2.0 calories. In the experiment on February 10, the basal value was high and though there was a slight increase in heat production in the first half hour after the ingestion of water, there were significant decreases in all the succeeding half hours, the maximum decrease being 1.9 calories; the net change in 4 hours was −9.5. It may be questioned whether 32.3 calories was the real basal value for this experiment in view of the marked decrease in all but one of the subsequent periods.

In the experiments with 150 cc. of water, the heat production decreased in comparison with the preceding basal heat production, whether the basal values were below or above the average basal value for all the experiments with water. On April 30, there were but slight changes from the basal value, 29.7 calories, throughout the experiment, and there was the small change of −2.2 calories in 4 hours. The high basal values on December 14 and May 24, 32.7 and 32.9 calories, were followed in all subsequent half hours by decreases, many of them marked, of which the maximum was 2.6 calories in the fourth hour on May 24. The total decrease in 4 hours in the two experiments was 11.7 and 12.3 calories. As in the experiment on February 10 with 100 cc. of water, however, the high basal values were a possible cause of these large decreases and it may be questioned whether they are actually significant. The basal value on January 11 was close to the average basal value, so that the decreases in this experiment seem significant.

Among the four experiments with 200 cc. of water there were two in which the basal value was high, namely, on January 4 and December 1. Nevertheless, there was on January 4 a rise in heat production in the first hour after the ingestion of water, a gradual falling off in the remaining half hours with no greater decrease than 1.4 calories and a net change of but −3.2 calories in 4 hours, and on December 1, an increase in practically all of the half hour periods after the ingestion of water and a total

TABLE IV
SUMMARY OF HEAT PRODUCTION AFTER INGESTION OF WATER. (Calories per half hour)

Date and apparatus[1]	Amount	Basal[2]	Half hours after ingestion of water.								
			1	2	3	4	5	6	7	8	9
1926	cc.										
Jan. 20 U	50	30.2	30.7	29.2	29.2	29.5	29.3	29.7	29.6	29.5	30.2
May 17 U	50	30.3	30.5	29.9	29.7	29.2	29.1	28.8	29.6	29.0[3]	
Feb. 15 G	50	32.2	32.8	32.7	32.4	32.5	32.2	32.0	32.1	32.4	32.2
May 28 G	50	30.5	30.7	30.4	30.3	30.2	30.0	29.9	30.1	30.3	
	Average	30.8	31.2	30.6	30.4	30.4	30.2	30.1	30.4	30.3	31.2
Jan. 15 U	100	29.5	29.3	30.2	28.9	29.2	30.4	30.1	29.4	29.5	29.7
Feb. 8 U	100	30.8	31.3	30.6	30.8	29.9	30.4	30.3	30.7	30.6	31.0
Feb. 10 G	100	32.3	32.7	31.4	31.3	30.7	30.9	30.5	30.4	31.0	30.6
Apr. 28 G	100	30.3	30.9	31.3	30.5	30.6	29.9	30.1	30.4	30.7	
	Average	30.7	31.1	30.9	30.4	30.1	30.4	30.3	30.2	30.5	30.4
Jan. 11 U	150	30.8	30.4	29.4	29.4	29.5	29.5	29.6	29.9	29.9	30.9[3]
Apr. 30 U	150	29.7	29.7	29.9	29.4	29.3	29.3	29.2	29.7	28.9[3]	
Dec. 14[4] G	150	32.7	32.5	31.6	31.3	31.3	30.6	30.6	30.9	31.1	31.2
May 24 G	150	32.9	32.0	30.4	31.2	30.3	30.7	31.9	32.2	32.2	
	Average	31.5	31.2	30.3	30.3	30.1	30.0	30.3	30.7	30.5	31.1
Jan. 4 U	200	33.6	34.5	34.0	32.4	32.6	33.2	33.8	32.9	32.2	32.8
May 12 U	200	29.7	30.6	29.8	30.8	30.4	30.3	30.3	30.5[3]		
Nov. 23[4] G	200	30.1	30.5	31.2	31.1	30.5	30.3	30.3	30.3	30.5	30.6
Dec. 1[4] G	200	33.5	34.5	34.3	34.2	33.5	33.9	33.8	34.3	34.2	34.0
	Average	31.7	32.5	32.3	32.1	31.8	31.9	32.1	32.0	32.3	32.5
Dec. 30[4] U	250	31.7	32.0	32.4	32.0	30.7	30.9	32.1	31.9	31.1	31.0[3]
Mar. 8 U	250	29.7	30.4	30.2	30.4	30.2	30.2	29.9	30.0[3]		
Nov. 16[4] G	250[5]	30.8	32.3	31.5	32.2	31.2	30.9	30.6	31.2	30.7	30.4
Dec. 23[4] G	250	30.1	30.1	30.6	30.0	30.6	29.9	30.3	30.0	30.1	30.7
May 19 G	250	31.7	32.7	32.5	32.1	32.2	31.7	32.2	31.7	31.4	
Sept. 16 G	250	31.8	32.9	32.4	32.1	32.0	32.2	32.5	32.4	32.7[3]	
	Average[6]	31.0	31.6	31.6	31.3	31.1	31.0	31.4	31.2	31.3	
Dec. 17 G	500	30.4	31.8	31.6	33.0	33.2	29.7	29.9	30.7[3]		

[1] U = Universal apparatus; G = Gasometer apparatus.
[2] Calculated to one half hour from five 10-minute periods.
[3] Calculated from results for less than a half hour (15 to 28 minutes).
[4] 1925.
[5] Temperature of the water on Nov. 16, 1925 was 16.2°C.
[6] Does not include results of experiment on Nov. 16, 1925.

increase of 4.7 calories in 4 hours. In the two experiments on May 12 and November 23, the basal values were about 3 per cent lower than the average basal value in all the experiments with water and in all the succeeding half hour periods there was a small increase in heat production, an average of 0.7 calorie per half hour on May 12 and 0.4 calorie on November 23. In this group of experiments, three out of four showed a definite increase in heat production as the result of the ingestion of 200 cc. of water at 37° C.

In the group of six experiments with 250 cc. of water, which includes the experiment on November 16, 1925 with water at 16.2° C. there were four (March 8, November 16, May 19 and September 16) which gave increases in heat production for all, or nearly all, of the half hour periods after the ingestion of water. The increases are small (maximum 1.5 calories for water at 37° C.) but they seem significant because they are so numerous and because the group with 200 cc. of water indicated the same tendency. There was a negative change in 4 hours in only one of the six experiments, that is, 0.5 calorie on December 30. It is a striking fact that 200 cc. and 250 cc. of water at 37° C. have an effect on the metabolism, which cannot be ascribed to low basal metabolism because it has been shown that increases in heat production in both groups bear no relation to the basal values. In the one experiment with 500 cc. of water, the basal value was not far from the general average. There was a significant increase in the heat production for the first four half hours after the ingestion of water and a total change of +7.1 calories in three and one half hours.

In general, then, heat production after the ingestion of water at 37° C. was increased when the quantities were at least 200 cc. Some of the experiments with amounts of water smaller than 200 cc. gave slight increases in the heat production, but in most instances there was little or no change. In those experiments in which there was a marked decrease, it followed for the most part a high basal heat production. This was particularly true on February 10 with 100 cc. of water and on December 14 and May 24 with 150 cc. of water. In all the subsequent experiments in the table, in which there was a high basal value, there occurred either a positive increase for the early portion of the experiment or an increase for all of the experiment.

Results of Control Experiments Without Water

To determine the course of the metabolism without water, six control experiments were made with the same subject, J. C., in the post-absorptive condition, three with the Universal respiration apparatus and three

by the gasometer method, the order, number, and length of the periods of measurement without water being the same as in the experiments with water. In these control experiments there was a tendency for the respiratory quotients to be lower in the half hours following the preliminary and the rest periods and for the metabolism of carbohydrate to be lower, whether the preliminary respiratory quotient was normal or high. The heat production, as averaged for the different half hours, showed more increase than decrease in comparison with the preliminary heat production, although in the greater number of individual half hours of the experiments there were decreases which were generally small. These results when the subject was given no water are similar to the results found in the experiments with the smaller amounts of water.

General Discussion

It has been shown in the preceding discussion that when the quantities of water are large enough, there is a distinct increase in the carbohydrate metabolized as compared with the basal value, and also in the heat production. There seems to be, however, no quantitative relation between the increase in carbohydrate metabolized and the increase in heat production. That is to say, the increase in heat production cannot be accounted for mathematically by the heat of combustion of the additional carbohydrate metabolized. In general when there is a fall in the carbohydrate metabolized there is also a fall in the heat production, but in some groups the fall in carbohydrate metabolized is much greater with respect to its energy content than is the fall in total heat production. For example, in 4 hours with 150 cc. of water there was a lowering of the carbohydrate metabolized by 5 grams, which would be approximately 19 calories. But there was a fall of only 9 calories in the heat production. The values given for heat production are calculated from the respiratory exchange, and therefore increases in heat production are real increases in total metabolism.

Lublin (1928) accounts for the increased metabolism after ingestion of water as due to the work performed by the kidneys. Grollman (1929), however, points out that after the ingestion of water the rise in oxygen consumption is rapid, but that the metabolic change does not run parallel to the diuresis. He believes that the diuresis and metabolic rise are not related, but that the latter results from a general stimulation of the metabolism of the body cells, which may be considered as due to the dilution of the internal environment of the cells by the ingested fluid. In none of our experiments was there a marked diuresis, and it is questionable

whether the increased metabolism is due primarily to increased kidney activity or is due, first, to the transfer of the fluid from the alimentary tract into the blood stream, from which subsequently it is either removed by the tissues or secreted by the kidneys.

The findings suggest strongly that the transfer of fluid in the body, even when at body temperature, involves expenditure of energy. Also, the results in general indicate that the greater the quantity of water, the greater the requirement of energy, although one group of experiments (150 cc.) gave anomalous results in that both the metabolism of carbohydrate and the heat production fell off markedly compared with the results obtained in the other groups. There is no satisfactory explanation for this exception. As will be seen in subsequent papers, the same sort of phenomenon occurred in the experiments with dextrose and with levulose.

Summary

A series of experiments was made with a human subject, in which the respiratory exchange in the post-absorptive state was measured for a period of one hour before, and for 3½ to 4½ hours after, the ingestion of 50 to 500 cc. of water at 37° C. A few control experiments without water, under conditions otherwise similar, were also carried out. The measurements of the metabolism were made by means of the Benedict Universal apparatus and by use of the gasometer method.

In the control experiments without water, and in the experiments with ingestion of water in amounts less than 200 cc., the course of the carbohydrate metabolism and the heat production changed little, if at all.

The ingestion of 250 cc. of water was followed by a slight, but definite increase in the metabolism of carbohydrate for a period of one hour (average 4 per cent), and with 500 cc. there was an increase (42 per cent) lasting two hours. Thus amounts of water over 200 cc. work for a definite increase in the metabolism of carbohydrate.

The heat production was slightly increased (2 per cent) for 1½ hours in experiments with 200 cc. of water, for two hours (1 per cent) with 250 cc. of water and for two hours (7 per cent) with 500 cc. of water at 37° C.

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THE GASEOUS EXCHANGE OF THE HUMAN SUBJECT II. AS AFFECTED BY SMALL QUANTITIES OF DEXTROSE

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IN CONNECTION with an earlier investigation in this Laboratory (Carpenter 1925) an experiment was reported on the effect of 30 grams of dextrose on the respiratory exchange. The change in the respiratory quotient was so large, that questions arose as to how small a quantity of dextrose would produce significant changes in the respiratory quotient and metabolism, whether the type of reaction would be the same with all quantities, and whether the increases in carbohydrate metabolized and heat produced would bear a definite relationship to the amounts of dextrose ingested.

Although the effect of dextrose upon the respiratory exchange has been reported many times, there are few studies with man in which small quantities (under 50 grams) have been used. Mason (1925) determined the respiratory quotient and calculated the heat production after the ingestion of 25 grams of dextrose. He found no rise above the basal respiratory quotient, but an increase of 10 per cent in the heat production at the end of one hour after ingestion of the sugar. Lublin (1926) reported one experiment with 30 grams and two experiments with 38 grams. In all three there was a rise in respiratory quotient and oxygen absorption within one-half to one hour. Lambie and Redhead (1929) had one experiment with a normal subject to whom 20 grams of glucose were given intravenously, and the gaseous exchange was measured. The maximum respiratory quotient was found twenty minutes after the beginning of injection.

The Metabolism as Affected by Small Quantities of Dextrose

The results of our measurements of the respiratory exchange before and after the ingestion of small amounts of dextrose in water are given in Tables I to IV. The values are averages per half hour for the respiratory quotient, carbohydrate metabolized and heat production during the basal period and during the successive half-hours after the dextrose was taken. The methods of studying the respiratory exchange were the same as in the previous communication (*This Jour.* 1930, II, 360.). The

TABLE I

SUMMARY OF RESPIRATORY QUOTIENTS AFTER INGESTION OF DEXTROSE

Date and apparatus[1]	Amount ingested		Basal[2]	Half hours after ingestion of dextrose.								
	Dextrose	Water		1	2	3	4	5	6	7	8	9
1926	grams	cc.										
May 26 U	5.05	50	0.85	0.86	0.86	0.87	0.87	0.87	0.86	0.85		
June 2 U	5.05	50	.81	.83	.83	.81	.82	.81	.81	.80	0.81	
Feb. 17 G	5.25	50	.81	.81	.81	.83	.82	.81	.81	.80	.80	0.78
Feb. 24 G	5.25	50	.79	.80	.80	.83	.80	.80	.80	.78	.81	.80
		Average	.82	.83	.83	.84	.83	.82	.82	.81	.81	
Apr. 26 U	10.1	100	.89	.91	.90	.89	.90	.89	.87	.85	.85	
Feb. 19 U	10.5	100	.81	.83	.80	.83	.80	.81	.81	.82	.79	.81
Mar. 1 G	10.5	100	.83	.82	.87	.88	.86	.85	.83	.83	.81	.81
Mar. 5 G	10.5	100	.83	.83	.84	.87	.86	.83	.82	.80	.83	.83
		Average	.84	.85	.85	.87	.86	.85	.83	.83	.82	.82
Mar. 26 U	15.5	150	.85	.86	.87	.91	.89	.88	.87	.85	.83	.82
Feb. 26 U	15.75	150	.87	.88	.91	.91	.88	.87	.88	.87	.86	.85
Apr. 23 G	15.5	150	.79	.79	.82	.83	.83	.81	.79	.79	.79	
Mar. 10 G	15.75	150	.83	.84	.88	.87	.87	.86	.84	.82	.82	.83
		Average	.84	.84	.87	.88	.87	.86	.85	.83	.83	.83
Mar. 3 U	21	200	.84	.87	.89	.90	.90	.88	.87	.85	.84	.83
Apr. 15 U	21	200	.83	.86	.86	.87	.87	.87	.87	.83	.84	.81
May 3 G	20	200	.81	.83	.87	.88	.87	.85	.83	.82	.81	
Mar. 15 G	21	200	.84	.86	.91	.90	.88	.89	.87	.85	.85	.83
		Average	.83	.86	.88	.89	.88	.87	.86	.84	.84	.82
Jan. 25 U	25	250	.89	.90	.91	.93	.92	.89	.90	.89	.89	.87[3]
Mar. 31 U	26	250	.81	.83	.87	.89	.85	.82	.84	.83	.82	.80[3]
Mar. 19 G	26	250	.86	.84	.89	.90	.90	.88	.85	.82	.83	.83
May 14 G	26	250	.82	.82	.87	.87	.86	.86	.83	.83	.81	.81[3]
		Average	.85	.85	.89	.90	.88	.86	.86	.84	.84	.83
Feb. 12 U	35.8	350	.85	.84	.87	.90	.95	.89	.88	.87	.89	.83[3]
Dec. 10 G	52	500	.80	.83	.89	.91	.90	.88	.89	.89	.82[3]	
Dec. 15 G	104	500	.81	.84	.90	.91	.90	.90	.94	.92		

[1] U—Universal apparatus; G—gasometer apparatus. [2] Average for 50 minutes. [3] Average for less than a half hour (15–28 minutes).

dextrose was Kahlbaum's purified grape sugar. This was weighed, dissolved in water, warmed to 37° C. and taken through a rubber tube; 25 cc. pure water being used as final rinse water taken in the same manner.

Respiratory Quotient and Metabolism of Carbohydrate

The respiratory quotients calculated by half hour periods are given in Table I for the Universal apparatus and the gasometer method. As in the series with small quantities of water, the basal quotient varied widely, ranging from 0.79 to 0.89, but in none were there such low quotients as are occasionally seen in the literature, *viz.*, under 0.75. The changes after the ingestion of dextrose vary with the initial basal quotient and with the quantity of dextrose given. The amount of carbohydrate metabolized before and after ingestion of dextrose is given in Table II. In calculating the non-protein respiratory quotient correction was made for protein, using an average of 0.38 gram of nitrogen per hour. During the basal hour the amounts of carbohydrate varied from 1.68 to 3.99 grams per half hour, and for the most part were over 2 grams.

In the 4 experiments with 5 grams in 50 cc. of water, there were in general during the first two hours slight increases in the respiratory quotient, the greatest occurring in the experiment on February 24 in the third half hour, 0.04. There was a positive increase in the carbohydrate metabolized in three of the experiments for the first two hours, and in two, through the third hour. The increase in carbohydrate burned was greatest with the lowest basal figure, that is, on February 24. The reverse rule, however, does not hold as the next greatest increase in any experiment occurs with the highest basal carbohydrate metabolized on May 26. The maximum average summation of increases[1] was 0.8 gram, equivalent to 16 per cent of the amount ingested.

Ten grams of dextrose in 100 cc. of water were given in 4 experiments. There was a rise in the respiratory quotient in the majority of the periods for the first two hours. In the experiment on March 1, in the third half hour, there was an increase of 0.05. The first two experiments show an irregularity in the carbohydrate metabolized from one half hour to the next. On March 1, with the exception of the first half hour, there was an increase in the carbohydrate metabolized during the first 2½ hours with a subsequent lowering during the last of the experiment, that is, from the

[1] By this expression is meant the greatest increase that can be obtained mathematically by adding the increases in *successive half* hours. The number of half hours so used will vary in the different experiments according to the duration of increases as well as the amount of dextrose ingested.

[1] U = Universal apparatus; G = gasometer apparatus. [2] Average for 50 minutes. [3] Average for less than a half hour (15–28 minutes).

TABLE II

SUMMARY OF CARBOHYDRATE METABOLIZED AFTER INGESTION OF DEXTROSE (Grams per half hour)

Date and apparatus[1]	Amount ingested		Basal[2]	Half hours after ingestion of dextrose								
	Dextrose	Water		1	2	3	4	5	6	7	8	9
1926	grams	cc.										
May 26 U	5.05	50	3.17	3.36	3.36	3.66	3.29	3.50	3.33	3.03		
June 2 U	5.05	50	2.39	2.61	2.53	2.08	2.51	2.13	2.12	1.92	2.38	
Feb. 17 G	5.25	50	2.44	2.32	2.21	2.73	2.38	2.14	2.18	1.98	1.97	1.57
Feb. 24 G	5.25	50	1.68	1.95	2.00	2.70	2.12	1.88	2.13	1.50	2.12	1.92
		Average	2.42	2.56	2.53	2.79	2.58	2.41	2.44	2.11	2.16	
Apr. 26 U	10.1	100	3.99	4.45	4.24	3.91	4.06	3.94	3.37	3.08	3.03	
Feb. 19 U	10.5	100	2.19	2.74	2.16	2.93	2.04	2.20	2.18	2.42	1.56	2.14
Mar. 1 G	10.5	100	2.76	2.62	3.84	3.91	3.17	2.95	2.62	2.67	2.30	2.30
Mar. 5 G	10.5	100	2.43	2.76	3.02	3.66	3.17	2.67	2.49	1.83	2.57	2.59
		Average	2.84	3.14	3.32	3.60	3.11	2.94	2.67	2.50	2.37	2.34
Mar. 26 U	15.5	150	3.01	3.48	3.56	4.48	3.86	3.71	3.35	2.90	2.62	2.49
Feb. 26 U	15.75	150	3.56	4.01	4.77	4.83	3.83	3.41	3.62	3.37	3.23	3.03
Apr. 23 G	15.5	150	1.79	1.87	2.47	2.45	2.68	2.10	1.74	1.97	2.03	
Mar. 10 G	15.75	150	2.70	3.09	4.14	3.84	3.64	3.44	2.86	2.53	2.59	2.82
		Average	2.77	3.11	3.74	3.90	3.50	3.17	2.89	2.69	2.62	2.78
Mar. 3 U	21	200	2.91	3.66	4.45	4.40	3.99	3.64	3.40	3.03	2.84	2.67
Apr. 15 U	21	200	2.71	3.52	3.83	3.76	3.60	3.71	3.69	2.78	2.96	2.22
May 3 G	20	200	2.40	3.06	4.10	3.73	3.84	3.22	2.61	2.68	2.52	
Mar. 15 G	21	200	3.13	3.74	5.03	4.50	3.80	4.03	3.66	3.31	3.17	2.79
		Average	2.79	3.50	4.35	4.10	3.81	3.65	3.34	2.95	2.87	2.56
Jan. 25 U	25	250	3.87	4.60	4.91	5.17	4.69	4.06	4.24	4.03	4.01	3.56[3]
Mar. 31 U	26	250	2.38	2.92	3.85	4.18	3.03	2.27	2.74	2.48	2.35	1.96[3]
Mar. 19 G	26	250	3.48	3.09	4.45	4.72	4.69	3.71	3.06	2.22	2.68	2.71
May 14 G	26	250	2.47	2.90	4.19	3.82	3.62	3.29	2.61	2.51	2.38	2.19[3]
		Average	3.05	3.38	4.35	4.47	4.01	3.33	3.16	2.81	2.86	2.61
Feb. 12 U	35.8	350	3.08	3.08	3.95	4.60	5.48	4.03	3.60	3.66	4.18	2.78[3]
Dec. 10 G	52	500	2.08	3.06	4.62	5.25	5.12	4.08	4.18	4.42	2.75[3]	
Dec. 15 G	104	500	2.13	3.33	5.31	5.20	5.01	4.85	6.16	5.58		

[1] U = Universal apparatus; G = Gasometer apparatus.

[2] Average of less than a half-hour (15 to 28 minutes).

[3] Calculated to one half hour from 5 ten-minute periods.

last half of the third hour on. All of the values during the first three hours on March 5 show an increase in the carbohydrate metabolized. The maximum average summation of increases was 1.9 grams, equivalent to 19 per cent of the dextrose ingested. The water alone had but little effect upon the carbohydrate metabolized.

Four experiments with 15 grams of dextrose resulted in an increase in the respiratory quotient for the first three hours in all but three of the periods. The maximum increase was 0.06 in the third half hour on March 26. Many of the other increases were as high as 0.04. There were increases in the carbohydrate metabolized during the first two hours and in two of the experiments, March 26 and March 10, the increase persisted throughout the third hour. The greatest increase in any one half hour occurs in the third on March 26, 1.47 grams. The maximum average increment was 3.7 grams, equivalent to 25 per cent of the dextrose given. The experiments with 150 cc. of water alone gave exceptional results in that the decrease in carbohydrate metabolized was larger than after 100 cc. of water. In three hours the average drop was 3.3 grams, so that the maximum net effect of 15 grams of dextrose would be an increase of 7.0 grams in the carbohydrate metabolized.

In the four experiments with 21 grams the increases in the respiratory quotient were more marked and there was a positive rise in the first three hours in every case, the maximum being 0.07 on May 3 and March 15. In these experiments the increases in carbohydrate metabolized were also more marked than in any of the preceding groups. All experiments gave increases in each period for the first three half hours; the increase continued in three of the experiments through the eighth half hour, that is, over a total of four hours. There was a fair degree of uniformity in the four experiments in the increase in carbohydrate metabolized for the first four hours, 6.1 to 6.6 grams, after the ingestion of 21 grams; this increase is equal to about 30 per cent of the carbohydrate ingested. There was no increase in carbohydrate metabolized due to 200 cc. of water alone, but rather a fall on the average of 3.9 grams in four hours. If this is added to the change due to 21 grams of dextrose, it would raise the results so that the average net change in 4 hours would be +10.2 grams. However, the basal carbohydrate metabolized averaged 3.41 grams per half hour in the water experiments and 2.79 grams in the experiments with dextrose. The correction for the change due to the ingestion of the water would therefore probably not be 3.9 grams but less.

In the experiments with 26 grams there was a positive rise in the respiratory quotient for the first three hours in all but four instances. In general,

* Average of less than a half-hour (15 to 28 minutes).

the rise in this group was not so high as with 21 grams. In two cases, however, the basal respiratory quotient was high, namely, January 25, 0.89, and March 19, 0.86. In the four experiments with 26 grams, there was increased metabolism of carbohydrate in practically all periods during the first three and one-half hours. The exceptions are the fifth half hour on March 31, and the first, sixth and seventh half hours on March 19. The maximum increase in any one half hour was on March 31, the third half hour, 1.8 grams. In general the greatest increases came in the second and third half hours. In the experiment on January 25, which has a high basal carbohydrate per half hour, 3.87 grams, the increases were continued throughout four hours, whereas with the slightly lower basal carbohydrate on March 19, there is a continuous decrease per half hour beginning with the sixth half hour. In general this group did not show so large an increase in the carbohydrate metabolized as the experiments with 21 grams, although the amount ingested here was 5 grams greater. The average increment in three hours was 4.4 grams, equivalent to 17 per cent of the sugar ingested. In this group the average basal metabolism of carbohydrate was 3.05 grams, whereas in the 21-gram group it was 2.79 grams, which may account in part for the lower increase with 25 grams. The increase in carbohydrate metabolized due to 250 cc. of water alone plays little or no rôle in the change due to ingestion of 25 grams of dextrose.

With 36 grams on February 12, in the fourth half hour, there was a rise of 0.10 in the respiratory quotient, and a rise in the quotient in all of the half hours with the exception of the first and ninth. There was an increase in the carbohydrate metabolized per half hour beginning with the second half hour after ingestion and continuing through the eighth. The total increase was 7.94 grams in four hours, thus equivalent to about one-fifth of the amount ingested. There was no experiment with 350 cc. of water alone. The experiments with 250 cc. gave an increase of 0.2 gram of carbohydrate in the first hour, and 500 cc. of water alone in one experiment resulted in an increase of 3.2 grams in 2 hours, so that about 1.5 grams of the rise after ingestion of 36 grams of dextrose may be ascribed to the water alone.

With 52 and 104 grams, there was a rise in the respiratory quotient of 0.10 in the second hour and a rise in all of the periods. The ingestion of 52 grams in 500 cc. of water produced increases in the carbohydrate metabolized in all the periods, the maximum occurring in the third half hour, 3.17 grams. During the four hours there was an increase of 16.8 grams in carbohydrate metabolized, which is equivalent to 32 per cent of the amount ingested. In the experiment with 104 grams, the increment was

20.5 grams in three and one-half hours, equivalent to 20 per cent of the dextrose ingested, but in neither of these experiments was the total increase in the metabolism of carbohydrate measured, as the amount per half hour did not fall to the basal level. The 500 cc. of water alone resulted in an increase of 3.2 grams in the carbohydrate metabolized, so that part of the rise due to 52 and 104 grams may be ascribed to the water of dilution.

The results show that, in general, quantities from 10 grams upwards of dextrose produce a positive rise in the respiratory quotient, beginning usually during the second half hour after ingestion, and the maximum quotient is obtained in the third half hour after ingestion. The average maximum rise in a single period for each group is shown in Figure 1.

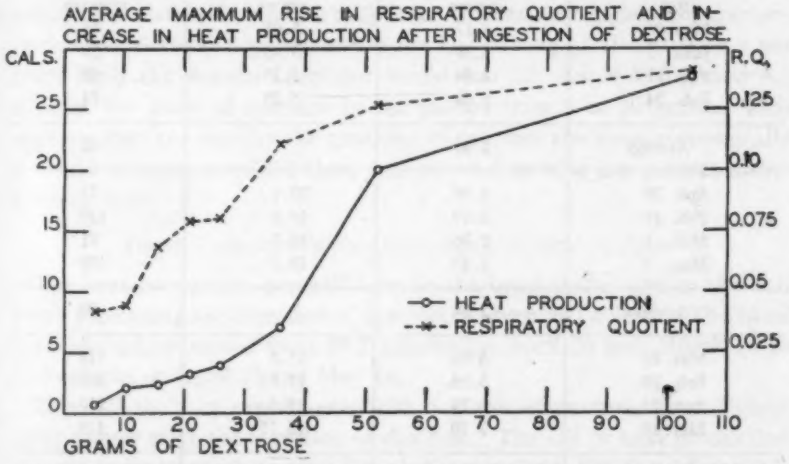

FIGURE 1. The upper line shows the average maximum increase in any single period for the respiratory quotient after the ingestion of dextrose. The lower line shows the average summation of increase in heat production above basal for each group.

It is evident that dextrose given in 10 gram amounts or over will raise the carbohydrate levels for a period of 1 hour or more, so that in order to supply carbohydrate equal to that metabolized by our subject in the basal condition dextrose should be given in amounts under 10 grams at intervals of one and one-half hours.

LENGTH OF TIME IN WHICH DEXTROSE INGESTED WOULD REPLACE THE CARBOHYDRATE METABOLIZED

The length of time in which the dextrose taken would replace the carbohydrate burned after the sugar is taken is given in Table III. In the

four experiments with 5.05 to 5.25 grams, the sugar ingested would last about an hour. The longest period is with the lowest basal carbohydrate of 1.68 grams on February 24, whereas the shortest period is with the highest basal carbohydrate of 3.17 grams on May 26. In the four experiments

TABLE III

LENGTH OF TIME DURING WHICH INGESTED DEXTROSE WOULD REPLACE THE CARBOHYDRATE METABOLIZED

Date	Basal carbohydrate per half hour	Dextrose ingested	Replaces sugar metabolized
1926	grams	grams	minutes
May 26	3.17	5.05	45
June 2	2.39	5.05	59
Feb. 17	2.44	5.25	68
Feb. 24	1.68	5.25	74
Average	2.42		62
Apr. 26	3.99	10.1	71
Feb. 19	2.19	10.5	129
Mar. 1	2.76	10.5	91
Mar. 5	2.43	10.5	100
Average	2.84		98
Mar. 26	3.01	15.5	121
Feb. 26	3.56	15.75	107
Apr. 23	1.79	15.5	213
Mar. 10	2.70	15.75	129
Average	2.77		143
Mar. 3	2.91	21	158
Apr. 15	2.71	21	171
May 3	2.40	20	174
Mar. 15	3.13	21	149
Average	2.79		163
Jan. 25	3.87	25	161
Mar. 31	2.38	26	267
Mar. 19	3.48	26	211
May 14	2.47	26	249
Average	3.05		222

with 10.1 to 10.5 grams, the basal carbohydrate is higher on the average, and the sugar ingested would last about one and one-half hours. The inverse relationship between the basal carbohydrate and the length of time in which the dextrose would replace the carbohydrate burned holds in the 10-gram series. In the experiments with 15.5 and 15.75 grams, the average duration as compared with the 10-gram group is increased by 45 minutes, which corresponds nearly to the difference in duration between the 5 and the 10 gram groups, *i.e.*, 36 minutes. The additional duration of carbohydrate metabolized, supplied by the dextrose when 20 to 21 grams are taken, is 20 minutes. The group of 25–26 grams would supply the carbohydrate metabolized for 222 minutes, an increase of 59 minutes due to the extra 5 grams. With the higher amounts of 36, 52, and 104 grams, the metabolism of carbohydrate is relatively higher per minute, so that the dextrose ingested would not last so long. Calculated on a per gram basis the dextrose ingested would last 12, 9.4, 9.2, 8.0, and 8.5 minutes per gram of dextrose in the groups from 5 to 26 grams. This suggests that the smaller the quantity of dextrose the more economically it would be used, provided there was no need to raise the general carbohydrate level.

Heat Production and Specific Dynamic Action[1]

The heat production per half hour in the basal period and in the half hours succeeding the ingestion of dextrose is given in Table IV. The basal heat production varied from 29.2 calories on April 26 and March 26 to 32.0 calories on April 15 and May 14.

Three of the four experiments with 5 grams of dextrose gave slightly lower values after the ingestion of dextrose. The fall in heat production was not quite so much as after 50 cc. of water alone, but the difference is not significant.

In the experiments with 10 grams, there were increases in the first hour in all four experiments, and on February 19, slight increases occurred successively through the fifth half hour. The maximum increase in any one half hour was 2.4 calories in the second on February 19. The experiments of February 19 and March 5 showed the greatest total increase, the maxi-

[1] Benedict and Carpenter (1918 p. 335) designated the relationship between the increase in heat production and the fuel value of the ingested material as the "cost of digestion." The expression "specific dynamic action" has also been used by some writers in the same sense, and as this phrase is the more commonly used as a synonym for the stimulating effect of food components upon total metabolism, the percentage relationship between the fuel value of the ingested sugar and the increase in heat production will be called "specific dynamic action" in this and the following article.

TABLE IV
SUMMARY OF HEAT PRODUCED AFTER INGESTION OF DEXTROSE (Calories per half hour)

Date and apparatus[1]	Amount		Basal[2]	Half hours after ingestion of dextrose								
	Dextrose	Water		1	2	3	4	5	6	7	8	9
1926	grams	cc.										
May 26 U	5.05	50	30.5	30.4	30.4	29.6	28.4	29.9	30.1	29.3		
June 2 U	5.05	50	30.9	31.0	30.3	29.7	30.0	30.3	30.2	30.2	30.8	
Feb. 17 G	5.25	50	31.7	32.8	31.5	30.4	31.0	30.7	31.1	31.3	31.2	31.5
Feb. 24 G	5.25	50	30.0	30.9	31.6	30.1	30.4	30.0	30.5	30.3	30.4	30.5
		Average	30.8	31.3	31.0	30.0	30.0	30.2	30.5	30.3	30.8	
Apr. 26 U	10.1	100	29.2	29.6	29.5	28.7	28.5	28.9	29.0	29.7	29.3	
Feb. 19 U	10.5	100	31.0	32.3	33.4	32.1	31.8	31.2	30.9	31.2	31.1	30.4
Mar. 1 G	10.5	100	30.7	31.4	31.1	29.0	29.2	29.0	29.4	29.9	30.2	30.2
Mar. 5 G	10.5	100	29.5	30.7	31.2	29.9	29.2	29.9	30.1	29.3	29.0	29.1
		Average	30.1	31.0	31.3	29.9	29.7	29.8	29.9	30.0	29.9	29.9
Mar. 26 U	15.5	150	29.2	29.7	30.3	29.7	28.4	28.7	28.8	28.3	29.2	29.8
Feb. 26 U	15.75	150	30.3	30.6	31.4	30.5	29.4	29.3	29.3	29.0	29.4	29.3
Apr. 23 G	15.5	150	31.6	32.7	32.0	29.6	30.0	30.2	30.8	31.2	31.9	
Mar. 10 G	15.75	150	30.1	31.8	33.1	31.1	29.7	29.7	29.8	30.5	31.1	31.3
		Average	30.3	31.2	31.7	30.2	29.4	29.5	29.7	29.8	30.4	30.1
Mar. 3 U	21	200	30.0	31.1	32.0	30.4	29.2	29.5	29.1	29.3	29.4	29.6
Apr. 15 U	21	200	32.0	33.3	33.9	31.8	30.6	29.9	29.8	30.6	30.4	31.3
May 3 G	20	200	31.3	33.5	32.8	30.3	31.1	31.1	31.2	32.0	32.6	
Mar. 15 G	21	200	30.4	31.9	31.8	30.1	29.5	29.7	29.9	30.2	30.7	31.0
		Average	30.9	32.5	32.6	30.7	30.1	30.1	30.0	30.5	30.8	30.6
Jan. 25 U	25	250	29.7	31.6	32.1	31.2	29.8	29.6	29.5	29.5	29.3	30.3[3]
Mar. 31 U	26	250	30.8	31.9	32.4	30.4	29.3	29.6	28.5	29.7	30.5	30.8[3]
Mar. 19 G	26	250	30.0	31.8	32.2	31.3	31.2	30.2	29.8	29.3	30.0	30.3
May 14 G	26	250	32.0	34.1	33.4	30.9	31.0	30.0	31.2	30.2	31.0	31.2[3]
		Average	30.6	32.4	32.5	31.0	30.3	29.9	29.8	29.7	30.2	30.7
Feb. 12 U	35.8	350	29.7	31.4	33.1	31.6	29.7	29.5	29.2	29.6	30.4	30.6[3]
Dec. 10 G	52	500	30.0	33.5	33.3	33.0	33.5	31.3	30.6	32.1	32.7[3]	
Dec. 15 G	104	500	30.5	36.0	36.0	34.0	34.2	33.3	34.1	33.5		

[1] U = Universal apparatus; G = Gasometer apparatus.
[2] Average of less than a half-hour (15 to 28 minutes).
[3] Calculated to one-half hour from 5 ten-minute periods.

mum being 6 calories on February 19, and 4 calories on March 5. These increases are equivalent to 16 and 11 per cent, respectively, of the heat value of the dextrose ingested, *i.e.*, specific dynamic action, but if the average increase during the first hour is used (2.1 calories), there was a 6 per cent specific dynamic action. With 100 cc. of water[1] alone the change was +0.5 calorie the first hour, and −0.5 calorie the second hour. The correction for water alone would affect but little the maximum increase after 10 grams of dextrose.

With 15.5 to 15.75 grams of dextrose, there was an increase in the heat production in all four experiments during the first two half hours. The maximum summation of increments by half hours was 5.7 calories on March 10, and the average increase in the first hour was 2.3 calories, equivalent to 4 per cent of the heat value of the ingested dextrose. The results of the experiments with 150 cc. of water alone were anomalous in that they showed greater drop in heat production than any other group, so that correcting the above values for the drop after water alone would raise materially the changes in heat production.

In four experiments with 20 and 21 grams, the increases occurred only during the first hour. After this, for the most part, the values were lower than those for basal. One experiment gave an increase of 4.2 calories in four hours, and maximum summation of increments occurred only in the first hour in all of them, averaging 3.2 calories, which is equivalent to 5 per cent as the specific dynamic action. The effect of 200 cc. of water was to increase the heat production by 2.6 calories in 4 hours. This would nearly wipe out any effect due to dextrose alone, and therefore would bring about the result that 21 grams of dextrose were not so effective in increasing the heat production as smaller amounts.

In four experiments with 25 and 26 grams, there was an increase in heat production during the first two hours on January 25 and March 19. On May 14 and March 31, the increase occurred mainly during the first hour. In general, the heat production was slightly greater after the ingestion of 25 grams than after 21 grams. The maximum summation of increases was 6.5 calories in two hours on March 19, and the average increase in two hours was 3.7 calories, equivalent to 4 per cent of the fuel value of the dextrose ingested. The heat production after 250 cc. of water alone was increased 1.2 calories the first hour, which would leave 2.4 calories as the increment in one hour due to 26 grams of dextrose alone.

The heat production in the experiment with 35.8 grams rose markedly in the first three half hours. The maximum increase was 3.4 calories in

[1] For the effect upon metabolism of ingestion of water alone, see previous paper, p. 359.

* Average of less than a half-hour (15 to 28 minutes).

the second half hour. The greatest increase summed to 7.0 calories in 2 hours, *i.e.*, 5 per cent specific dynamic action. No experiments were made with 350 cc. of water alone, but one may interpolate the results between 250 and 500 cc. This would give as the increase in heat production during the first hour due to water alone 1.8 calories, which would leave 3.3 calories due to the dextrose alone. It is evident that some of the increase in heat production after 350 cc. of 10 per cent solution of dextrose may be due to water alone.

With 52 grams, the maximum increase was 3.5 calories in the first and fourth half hours, and the minimum increase was 0.6 calorie in the sixth half hour. Throughout the first two hours there was an increase of 10 per cent or over. The increase in four hours was 20.0 calories, and this apparently does not include the total increase due to 52 grams of dextrose, as the heat production had not reached basal at the end of four hours. The difference between 36 grams and 52 grams is 16, and this additional amount caused a greater additional increase in heat production than any quantity under 36 grams.

With 104 grams, there was an increase of 5.5 calories in each of the first two half hours, *i.e.*, 18 per cent above the basal heat production. The experiment was only three and one half hours in length, and during this time there was an increase of 27.6 calories, *i.e.*, 13 per cent above basal, but this does not include all the heat increase due to 104 grams, as the heat production had not reached basal during the 3 1/2 hours.

With 52 grams, the increase of 20 calories equals 10.3 per cent as the specific dynamic action and with 104 grams, the increase of 27.6 calories in three and a half hours equals 7.1 per cent, but in neither of these was the duration long enough to measure all of the heat increment. Benedict and Carpenter (1918) found with 75 and 100 grams of dextrose from 2 to 9 per cent, and Deuel (1927) found 8 per cent with 75 grams of glucose.

Only one experiment with 500 cc. of water alone was made, and this resulted in an increase of 8.0 calories in 2 hours. The subtraction of this amount from the increases of 13.3 and 18.2 calories after 52 and 104 grams would lower materially the increase due to dextrose alone. It is evident that further investigation is necessary to determine whether there is a real difference in heat production as affected by sugars given dry or given in 10 per cent solution. This situation raises the question as to how much of the effect of food is due specifically to the food itself and how much to the fluids taken along with it.

Apparently there is a critical level in the relationship between the energy value of dextrose and the increment of heat production after the ingestion

of a 10 per cent solution of this sugar. This level occurs with amounts of dextrose up to and including 25 grams; with these amounts the increment in heat production equals about 4 per cent of the energy value of the dextrose. Above 25 grams the increase in heat production is greater. The average summated increase in heat production for each group is shown in Figure 1, page 381.

This finding is of value where it is desirable to give dextrose without raising noticeably the heat production. It is evident that 25 grams or under may be given at intervals of one and a half hours without causing a rise in the heat production of over 6 per cent. Such a procedure would be useful when one wishes to supply carbohydrate just sufficient to be above the level of ketogenesis, but does not want to produce glycosuria. Amounts above 25 grams would lead to greater increases in heat production which in turn would require a greater energy supply for maintenance. It should be noted that 25 grams far exceeds the maximum requirements for carbohydrate supply for one and a half hours. The greatest carbohydrate use in the first three half hours after dextrose ingestion in these experiments was 14.7 grams on January 25 when 25 grams were taken. An amount between 10 and 15 grams would be equivalent to the sugar used in one and a half hours with this subject. The amount to be taken would also depend on how low a carbohydrate level the subject had, when the ingestion began.

Summary

The respiratory exchange of a human in the post-absorptive condition was determined by two different methods, for from three to four hours after the ingestion of dextrose in amounts between 5 and 104 grams in water at 37° C.

In the majority of the observations, the maximum respiratory quotient was observed in the third half hour, and all amounts of 10 grams or over were accompanied by significant changes in the respiratory quotient.

Increases in the metabolism of carbohydrate were observed in the first one and one-half hours with amounts of 5 to 10 grams, in 3 hours with 15 grams and in three and one-half to four hours with 21 to 25 grams.

The increase in carbohydrate metabolism above basal for 3 hours represented on the average from 17 to 31 per cent of the amount of dextrose ingested in quantities of 10 to 36 grams.

The increase in heat production was practically 1 calorie for each additional 5 grams from 5 to 25 grams. The increase in the heat production above 25-gram amounts rose more rapidly in proportion to the additional amounts given.

The specific dynamic action, that is, the relation of the increase in heat production to the energy value of the ingested dextrose, varied from 4 to 6 per cent when 5 to 36 grams were given. When 52 grams were taken, the value was 10 per cent.

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THE GASEOUS EXCHANGE OF THE HUMAN SUBJECT III. AS AFFECTED BY SMALL QUANTITIES OF LEVULOSE

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IN A PREVIOUS investigation in this Laboratory (Carpenter 1925), an experiment with 30 grams of levulose in 500 cc. of water was reported in which the respiratory exchange was measured. There was a marked rise in the respiratory quotient, which was nearly as large as that observed in an earlier study (Benedict and Carpenter 1918) with the much larger quantities of 75 and 100 grams. This result indicated the desirability of experiments with small quantities of levulose in order to determine the minimum quantity that would produce a measurable change in the respiratory quotient, and in the metabolism as represented by the calculated heat production. Also, in view of the characteristic course of the respiratory quotient after comparatively large amounts of levulose as compared with that after dextrose, the question arose as to whether this typical curve would occur when the quantities were much smaller.

The number of experiments in which levulose has been given to man in quantities under 50 grams, and the respiratory exchange measured, is relatively small. Lublin (1926) reported two experiments with 20 grams and three experiments with 30 grams of levulose. The respiratory quotient rose markedly within an hour in all of the experiments and there was also an increase in the consumption of oxygen.

The methods of measuring the respiratory exchange before and after the ingestion of levulose were the same in the experiments here reported as in the preceding studies on the effect of the ingestion of water and of small quantities of dextrose. (*This Jour.* 1930, II, 359 and 375.) The levulose used was Kahlbaum's commercial grade of levulose, which was weighed out as needed, dissolved in pure water and warmed (in all but one experiment) to 37°C. The solution was usually taken by sucking it through a small rubber tube and using 25 cc. of water for final rinsing of the container and tubing.

Results of Ingestion of Levulose

Basal values and results in half hours following the ingestion of the levulose are given for the respiratory quotient, carbohydrate metabolized

and heat produced in all the experiments with the gasometer method and the Universal apparatus. As in the preceding two papers, the first of the basal periods was rejected and the basal values given were derived from the average of periods 2 to 6 inclusive, in the basal hour preceding the ingestion of sugar.

As the respiratory quotient is computed from the total respiratory exchange, the metabolism of protein must be obtained for the calculation of the total heat produced and consequently for the carbohydrate metabolized. These computations have been carried out for the basal period and for the half hours after the ingestion of levulose in the same manner as in the experiments with water and with dextrose, the average urinary nitrogen per hour in the experiments with levulose being 0.43 gram for the experiments with the Universal apparatus and 0.39 gram for the experiments with the gasometer apparatus.

Respiratory Quotient and Metabolism of Carbohydrate

The respiratory quotients and carbohydrate metabolized, are given for the experiments with levulose in Tables I and II. In general, the ingestion of 5 grams of levulose was followed by a slight rise in the respiratory quotient within an hour. The metabolism of carbohydrate was definitely increased in the first half hour of five of the experiments with 5 grams of levulose. On April 16 and February 5 this increase continued to the fourth half hour, and on the latter date through the ninth half hour. In the experiment on June 14, there was an increase lasting for one hour. The average increase in carbohydrate metabolized in the first half hour after 5 grams of levulose was 0.4, and in the second half hour, 0.3 gram.

On the days when 10 grams of levulose were ingested, there was a definite rise in the respiratory quotient in the second half hour in all the experiments and in the first half hour in three experiments. In general, the increase in quotient in the second half hour of the four experiments with this amount of levulose was greater than the increase after 5 grams of levulose. The carbohydrate metabolized was increased in all of the four experiments. The average increase in half hours 1 to 4 was 0.9, 1.7, 0.4, and 0.2 gram, respectively. The average summation[1] of increases for 2 hours in the four experiments was 3.1 grams.

In the experiments with 15.5 grams of levulose, there was an increase in

[1] By this expression is meant the greatest increase that can be obtained mathematically by adding the increases in *successive half* hours. The number of half hours so used will vary in the different experiments according to the duration of increases as well as the amount of levulose ingested.

the respiratory quotient lasting on January 8 throughout the 4½ hours of measurement, on March 22 for 3 hours, and on January 27 and March 29 for 1½ hours. There was thus a difference between the results obtained with the Universal apparatus on January 8 and March 22 and those obtained with the gasometer apparatus on January 27 and March 29. The increase in respiratory quotient during 1½ hours in these experiments with 15.5 grams was greater than that after either 5 or 10 grams of levulose. The metabolism of carbohydrate was increased immediately following the ingestion of levulose in all of the four experiments, but this increase varied in duration in the several experiments, lasting from 1½ hours to 4½ hours. The greatest increases occurred in the second half hour, in which the maximum was 4.06 grams on March 29. The average summation of increases for 3 hours in the four experiments was 8.2 grams.

Following the ingestion of 21 grams of levulose, there was a marked increase in respiratory quotient in the second half hour in all four experiments, and in the first half hour, in three of the experiments. The increases in the quotients lasted from 2 to 3 hours and were, in general, somewhat more sustained than in the preceding groups with levulose. The metabolism of carbohydrate was increased in all cases for at least 2 hours; on March 17, 2½ hours and on January 2 and November 27, 3 hours. The average summation of increases for 3 hours in the four experiments was 7.2 grams. Compared with the increases for the 15.5-gram group, the increases after 21 grams of levulose were mostly smaller, both by successive half hours and as summation of increases. The average basal carbohydrate was somewhat lower in the 15.5-gram group than in the 21-gram group, *i.e.*, 2.30 as compared with 3.06 grams. This fact may account for the higher increases after 15.5 grams of levulose.

In all of the six experiments with 26 grams of levulose, there was a definite increase in the respiratory quotient in the first half hour, and in five of the experiments these increases lasted from 2 to 3½ hours. The quotients were more sharply increased in the first hour than in the same length of time in the 21-gram group. There were increases in carbohydrate metabolized for from 2 to 3½ hours in these experiments with 26 grams, the maximum increase in any half hour being 3.8 grams in the second half hour on November 19. The average summation of increases for 3 hours in the six experiments was 7.8 grams. These changes in carbohydrate metabolized are of the same order of magnitude as those noted after the ingestion of 21 grams of levulose.

In one of the two experiments with 52 grams, the rise in respiratory quotient was 0.17 in the second half hour, which was equalled in the

TABLE I
SUMMARY OF RESPIRATORY QUOTIENTS AFTER INGESTION OF LEVULOSE

Date and apparatus[1]	Amount ingested		Basal[2]	Half hours after ingestion of levulose.								
	Levulose	Water		1	2	3	4	5	6	7	8	9
1926	grams	cc.										
Jan. 18 U	5.05	50	0.89	0.91	0.87	0.83	0.85	0.85	0.86	0.85	0.84	0.83
Jan. 22 U	5.05	50	.88	.91	.88	.87	.87	.86	.87	.87	.84	.84[3]
Feb. 5 G	5.05	50	.79	.81	.82	.82	.81	.79	.82	.81	.81	.80
Apr. 16 G	5.05	50	.83	.83	.86	.85	.84	.81	.81	.81	.81	.81
June 9 G	5.05	50	.84	.81	.84	.81	.79	.80	.81	.81	.80	
June 14 G	5.05	100	.81	.86	.83	.80	.81	.83	.83	.80	.80[3]	
		Average	.84	.86	.85	.83	.83	.82	.83	.83	.82	.82
Jan. 13 U	10.1	100	.88	.89	.96	.90	.88	.89	.87	.85	.86	.83
Apr. 21 U	10.1	100	.82	.83	.86	.83	.82	.82	.83	.82	.81	.81
Feb. 1 G	10.1	100	.83	.91	.89	.84	.85	.84	.83	.81	.82	.83
Apr. 12 G	10.1	100	.80	.77	.87	.83	.81	.80	.81	.79	.80	.80
		Average	.83	.85	.90	.85	.84	.84	.84	.82	.82	.82
Jan. 8 U	15.5	150	.81	.89	.95	.86	.87	.86	.87	.84	.83	.85[3]
Mar. 22 U	15.5	150	.81	.84	.94	.88	.86	.85	.84	.81	.80	.82[3]
Jan. 27 G	15.5	150	.85	.92	.96	.87	.83	.80	.81	.83	.81	.82
Mar. 29 G	15.5	150	.80	.86	.95	.86	.81	.81	.81	.80	.80	.81
		Average	.82	.88	.95	.87	.84	.83	.83	.82	.81	.82
Jan. 2 U	21	200	.83	.87	.95	.91	.89	.85	.86	.82	.84	.85
Mar. 17 U	21	200	.83	.85	.93	.92	.88	.89	.83	.82	.81	.81[3]
Nov. 27[4] G	21	200	.84	.83	.90	.92	.87	.86	.86	.84	.85	.84
Dec. 4[4] G	21	200	.87	.92	.96	.91	.89	.86	.86	.82	.83	.83
		Average	.84	.87	.94	.92	.88	.87	.85	.83	.83	.83

TABLE I. CONT'D

Dec. 28[4]	U	26	250	.94	1.02	1.00	.90	.93	.94	.90	.92	.89	.89
Mar. 13	U	26	250	.83	.89	.98	.92	.90	.86	.87	.86	.83	.83[3]
Nov. 19[4]	G	26	250[5]	.81	.85	.94	.91	.91	.83	.81	.82	.79	.80
Dec. 17[4]	G	26	250	.85	.88	1.02	.94	.89	.86	.85	.83	.84	.85
Mar. 24	G	26	250	.80	.83	.93	.89	.83	.84	.82	.79	.79	.81
Sept. 23	G	26	250	.88	.92	1.00	.98	.90	.87	.86	.84	.83[3]	
			Average	.85	.90	.98	.92	.89	.87	.85	.84	.83	.84
Dec. 8	G	52	500	.80	.92	.88	.88	.88	.84	.83	.88[3]		
Dec. 22	G	52	500	.81	.89	.98	.90	.87	.87	.86	.81		
			Average	.81	.91	.93	.89	.88	.86	.85	.85		
Dec. 13	G	104	500	.84	.98	1.01	.98	1.00	.96	.92	.89	.92[3]	

[1] U = Universal apparatus; G = Gasometer apparatus.
[2] Average respiratory quotient for five 10-minute periods.
[3] Respiratory quotient for less than a half hour (15 to 29 minutes).
[4] 1925.
[5] The water in the experiment of Nov. 19, 1925 was at 16.8°C.

TABLE II
SUMMARY OF CARBOHYDRATE METABOLIZED AFTER INGESTION OF LEVULOSE (Grams per half hour).

Date and apparatus[1]	Amount ingested		Basal[2]	Half hours after ingestion of levulose								
	Levulose	Water		1	2	3	4	5	6	7	8	9
1926	grams	cc.										
Jan. 18 U	5.05	50	4.24	4.75	3.50	2.63	2.97	3.06	3.21	3.03	2.89	2.65
Jan. 22 U	5.05	50	3.88	4.50	3.87	3.69	3.44	3.37	3.46	3.44	2.60	2.77[3]
Feb. 5 G	5.05	50	1.54	2.20	2.65	2.34	2.05	1.65	2.32	2.08	1.88	1.88
Apr. 16 G	5.05	50	2.55	2.68	3.58	3.28	2.76	2.13	2.16	2.14	2.14	2.20
June 9 G	5.05	50	2.96	2.33	3.03	2.28	1.83	2.05	2.24	2.20	1.79	
June 14 G	5.05	100	2.26	3.46	2.58	1.91	2.15	2.82	2.58	1.97	1.98[3]	
		Average	2.91	3.32	3.20	2.69	2.53	2.51	2.66	2.48	2.21	2.38
Jan. 13 U	10.1	100	3.69	3.86	5.72	4.42	3.84	3.74	3.58	2.97	3.19	2.68
Apr. 21 U	10.1	100	2.43	2.92	3.54	2.56	2.38	2.59	2.82	2.31	2.14	2.12
Feb. 1 G	10.1	100	2.56	4.99	4.35	2.67	2.96	2.94	2.76	2.08	2.27	2.70
Apr. 12 G	10.1	100	2.03	2.36	3.87	2.59	2.22	1.77	2.18	1.59	1.85	1.77
		Average	2.68	3.53	4.37	3.06	2.85	2.76	2.84	2.24	2.36	2.32
Jan. 8 U	15.5	150	2.08	4.25	5.97	3.46	3.40	3.21	3.33	2.74	2.57	2.88[3]
Mar. 22 U	15.5	150	2.07	3.06	5.81	3.64	3.21	2.91	2.86	2.02	1.87	2.26[3]
Jan. 27 G	15.5	150	3.13	5.75	6.83	3.97	3.06	1.91	2.38	2.84	2.31	2.54
Mar. 29 G	15.5	150	1.92	3.48	5.98	3.36	2.07	2.04	2.07	1.74	1.87	2.07
		Average	2.30	4.14	6.15	3.61	2.94	2.52	2.66	2.34	2.16	2.44
Jan. 2 U	21	200	2.68	4.06	6.21	5.19	4.54	3.26	3.62	2.42	3.01	3.24
Mar. 17 U	21	200	2.49	3.04	4.93	4.55	3.78	3.94	2.56	2.19	1.83	1.97[3]
Nov. 27[4] G	21	200	3.19	3.09	4.64	5.47	3.97	3.67	3.62	3.14	3.46	2.92
Dec. 4[4] G	21	200	3.87	5.48	6.45	4.96	4.17	3.54	3.48	2.59	2.53	2.56
		Average	3.06	3.92	5.56	5.04	4.12	3.60	3.32	2.59	2.71	2.67

TABLE II. CONT'D

Dec. 28[4]	U	26	250	5.14	6.12	6.34	4.72	5.08	5.71	4.42	4.55	3.98	3.83
Mar. 13	U	26	250	2.62	4.13	6.01	4.81	4.27	3.37	3.60	3.19	2.62	2.42[3]
Nov. 19[4]	G	26	250[5]	2.14	3.01	5.94	4.74	4.66	2.70	2.15	2.39	1.76	1.97
Dec. 17[4]	G	26	250	3.06	3.99	6.59	5.60	4.17	3.39	3.14	2.52	2.71	3.02
Mar. 24	G	26	250	1.92	2.90	5.28	4.08	2.52	2.73	2.32	1.48	1.73	2.18
Sept. 23	G	26	250	3.71	5.14	6.48	6.37	4.55	3.66	3.35	2.84	2.68[3]	
			Average	3.10	4.22	6.11	5.05	4.21	3.59	3.16	2.83	2.58	2.68
Dec. 8	G	52	500	2.13	5.70	4.52	4.49	4.19	3.28	2.85	4.34[3]		
Dec. 22	G	52	500	2.21	5.05	7.16	5.09	3.95	4.05	3.73	2.11		
			Average	2.17	5.38	5.84	4.79	4.07	3.67	3.29	3.23		
Dec. 13	G	104	500	2.79	7.16	7.73	7.77	7.77	7.42	5.90	4.93	5.70[3]	

[1] U = Universal apparatus; G = Gasometer apparatus
[2] Calculated to one half hour from five 10-minute periods.
[3] Calculated from results for less than a half-hour (15 to 29 minutes).
[4] 1925.
[5] The water in the experiment of Nov. 19, 1925 was at 16.8°C.

preceding groups only in the second half hour after 26 grams on December 17. The increase in the respiratory quotient and the carbohydrate metabolized lasted 3½ hours in one experiment and three hours in the other. The two experiments gave total increases in carbohydrate metabolized of 14.5 and 15.7 grams, respectively, in 3½ hours, which are twice as great as those after half as much levulose, but we cannot be certain that all of the increase in carbohydrate was measured in the experiments with 52 grams, as the experiments were continued only 3½ hours after the ingestion of levulose, and the carbohydrate metabolized was still above the basal amount in the last half hour on December 8.

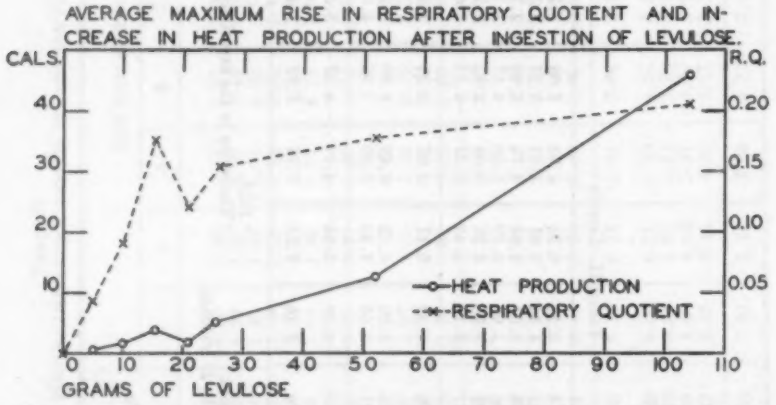

FIGURE 1. The upper line shows the average maximum increase in any single period for the respiratory quotient after the ingestion of levulose. The lower line shows the average summation of increase in heat production above basal for each group.

After the ingestion of 104 grams of levulose, there was a marked increase in the respiratory quotient which lasted at least the 4 hours of the observations. The carbohydrate metabolized did not return to the basal value in 4 hours. The total increase in 4 hours was 32.1 grams, which was twice as large as with one half the quantity of levulose, *i.e.*, 52 grams, but the experiment was not long enough to measure all the increase in carbohydrate.

The average of the maximum rise of the respiratory quotient in each experiment is shown for each group in Figure 1.

In general, there was a slight increase in carbohydrate metabolized after the ingestion of the smaller amounts of levulose, which rises with the amount ingested till the levulose is 15.5 grams. For 15.5 to 26 grams of

levulose, there is a plateau in the total increase of carbohydrate metabolized at about 7 to 8 grams, and above 26 grams, the increase in carbohydrate is at least proportional to the additional amounts given and may rise more rapidly than the increase in amount given.

Relation of Increase in Carbohydrate Metabolized to Levulose Ingested

In the experiments with 5 grams of levulose there was an average summation of increases of 0.7 gram in carbohydrate metabolized, equivalent to 14 per cent of the ingested levulose. For 10 to 104 grams of levulose, the increase measured was equal to from 29 to 34 per cent of the levulose ingested, except for 15.5 grams of levulose which resulted in an increase in carbohydrate metabolized equivalent to 53 per cent. It is probable that in the last three experiments the total increase was not measured, so that the real percentages represented by the increases after 52 and 104 grams of levulose would be somewhat higher than those given above. In general, these percentages are somewhat higher than those found in the experiments with small quantities of dextrose.

Length of Time in Which Levulose Ingested Would Replace Carbohydrate Metabolized.

The length of time in which the ingested levulose would replace the carbohydrate metabolized after the levulose was taken is given in Table III. In the six experiments with 5 grams of levulose there was a wide range in the basal carbohydrate and the extremes in duration of replacement bear inverse relationships to this range. Doubling the amount ingested to 10 grams increases, on the average, the duration of levulose as the supply by about 80 per cent. Again the maximum duration accompanies the lowest basal carbohydrate, and, *vice versa*, the minimum duration accompanies the highest carbohydrate. The addition of 5.4 grams of levulose, raising the amount ingested to 15.5 grams, increases the length of time the supply would last to 109 minutes on the average, *i.e.*, an increase of 23 minutes. The average for the 21-gram group is 31 minutes longer than that for the 15.5-gram group, an increase slightly greater than the difference in duration for the 10-gram and 15.5-gram groups. In the 26-gram group, the sugar would replace the carbohydrate metabolized for over three hours, or an additional 49 minutes over the average for the 21-gram group. The inverse relationship between high basal carbohydrate and shorter duration of the supply holds for the most part in the individual experiments. The larger amounts of 52 and 104 grams would replace the carbohydrate

TABLE III

LENGTH OF TIME IN WHICH LEVULOSE INGESTED WOULD REPLACE CARBOHYDRATE METABOLIZED

Date	Basal carbohydrate metabolized per half hour	Levulose given	Duration of replacement
1926	grams	grams	mins.
Jan. 18	4.24	5.05	33
" 22	3.88	"	34
Feb. 5	1.54	"	63
April 16	2.55	"	50
June 9	2.96	"	57
June 14	2.26	"	48
Average	2.91		48
Jan. 13	3.69	10.1	64
April 21	2.43	"	104
Feb. 1	2.56	"	69
April 12	2.03	"	107
Average	2.68		86
Jan. 8	2.08	15.5	106
Mar. 22	2.07	"	118
Jan. 27	3.13	"	82
Mar. 29	1.92	"	129
Average	2.30		109
Jan. 2	2.68	21	129
Mar. 17	2.49	"	159
Nov. 27[1]	3.19	"	151
Dec. 4[1]	3.87	"	120
Average	3.06		140
Dec. 28	5.14	26	140
Mar. 13	2.62	"	178
Nov. 19[1]	2.14	"	217
Dec. 11[1]	3.06	"	172
Mar. 24	1.92	"	281
Sept. 23	3.71	"	148
Average	3.10		189
Dec. 8	2.13	52	208[3]
Dec. 22	2.21	"	210[3]
Dec. 13	2.79	104	231[4]

[1] 1925. [3] 29.4 grams of carbohydrate. [3] 31.1 grams of carbohydrate. [4] 54.4 grams of carbohydrate.

metabolized for a somewhat longer period of time than the experiments lasted, as on the average only 30.3 grams of carbohydrate were metabolized in 209 minutes after the ingestion of 52 grams, and 54.4 grams of carbohydrate after the ingestion of 104 grams. Beginning with the 15.5 grams of levulose the increases in the duration of replacement of the carbohydrate burned are greater than would be expected from the additional amounts of levulose ingested.

Heat Production

The values for the heat production in the basal periods and in the half hours after the ingestion of levulose, are given in Table IV. In five of the six experiments in the 5-gram group, there was an increase in the first half hour, and in four, there was an increase in the second half hour. Beginning with the third half hour, the changes were mostly in the minus direction. The average change in heat production during 1 hour was +0.5 calorie.

In three of the experiments with 10 grams there was an increase in heat production in the first half hour, and in all four an increase in the second half hour. The average increase in the first half hour in this group was 1.0 calorie; in the second, 0.9 calorie. The average fall in this group, from half hour to half hour, beginning with the third half hour, was not so large as in the group with 5 grams of levulose. The average increase in 2 hours was 1.1 calories in contrast to the decrease in 2 hours found with the 5-gram group.

There were positive increases in the first two half hours in all four experiments with 15.5 grams, which ranged from 1.0 to 3.6 calories. In the experiment on March 22, there was practically no change after the first hour. Although there was a high basal value on January 27, the heat production was increased in each succeeding half hour. The average increase for the four experiments amounted to 1.9 calories both in the first and in the second half hours, and there was a slight increase, 0.3 calorie on the average, in the third half hour. The average increase in 3 hours after 15.5 grams of levulose was 4.0 calories. The 15.5-gram group as a whole gave a greater increase in heat production than the two preceding groups.

Three out of four experiments with 21 grams, namely those with the high basal heat production, gave increases in the first and second half

TABLE IV
SUMMARY OF HEAT PRODUCED AFTER INGESTION OF LEVULOSE (Calories per half hour).

Date and apparatus[1]	Amount ingested		Basal[2]	Half hours after ingestion of levulose								
	Levulose	Water		1	2	3	4	5	6	7	8	9
1926	grams	cc.										
Jan. 18 U	5.05	50	30.3	30.9	30.7	30.1	29.7	30.4	30.0	30.1	30.6	30.3
Jan. 22 U	5.05	50	31.9	30.7	30.5	30.6	30.2	29.8	30.4	30.2	29.8	29.6[3]
Feb. 5 G	5.05	50	30.9	31.4	31.7	30.6	29.7	29.5	30.4	30.0	30.0	30.0
Apr. 16 G	5.05	50	30.7	32.0	32.2	31.6	30.7	30.5	31.0	30.7	30.7	31.4
June 9 G	5.05	50	32.6	33.0	33.2	32.4	32.3	32.2	31.8	31.4	31.6	
June 14 G	5.05	100	32.1	33.0	30.9	30.3	30.8	31.3	30.9	31.2	31.3[3]	
		Average	31.4	31.8	31.5	30.9	30.6	30.6	30.8	30.6	30.7	30.3
Jan. 13 U	10.1	100	29.3	29.2	30.7	30.2	29.1	28.5	29.8	29.7	29.9	30.5
Apr. 21 U	10.1	100	32.2	32.7	32.5	31.4	31.6	31.6	31.9	30.9	31.2	30.9
Feb. 1 G	10.1	100	30.8	32.8	31.6	29.9	30.6	30.5	30.7	30.0	29.9	30.1
Apr. 12 G	10.1	100	31.9	33.3	32.8	31.1	31.7	31.3	31.1	31.8	32.4	31.3
		Average	31.1	32.0	31.9	30.7	30.8	30.5	30.9	30.6	30.9	30.7
Jan. 8 U	15.5	150	30.5	31.6	31.8	30.4	29.9	30.0	29.5	29.3	29.5	28.9[3]
Mar. 22 U	15.5	150	30.4	32.1	32.0	30.3	30.0	30.8	30.3	29.8	30.4	30.3[3]
Jan. 27 G	15.5	150	32.1	35.7	35.0	33.5	33.5	33.4	33.6	33.6	32.7	32.8
Mar. 29 G	15.5	150	30.5	31.5	32.2	30.6	29.8	29.5	29.8	30.8	29.9	29.8
		Average	30.9	32.7	32.8	31.2	30.8	30.9	30.8	30.9	30.6	30.5
Jan. 2 U	21	200	32.5	33.1	33.8	33.3	33.4	32.0	31.5	32.0	31.6	31.8
Mar. 17 U	21	200	30.6	30.2	29.8	29.8	29.9	29.7	29.4	29.5	29.9	29.2[3]
Nov. 27[4] G	21	200	32.7	33.8	33.4	34.2	33.5	33.0	32.5	32.3	33.0	32.2
Dec. 4[4] G	21	200	32.8	34.2	33.4	32.6	31.9	31.9	31.5	31.1	30.5	30.8
		Average	32.2	32.8	32.6	32.5	32.2	31.7	31.2	31.2	31.3	31.0

TABLE IV CONT'D

Dec. 28[4] U	26	250	29.9	31.6	32.5	31.9	30.6	31.6	30.2	29.8	30.0	30.2
Mar. 13 U	26	250	30.0	30.8	31.1	30.2	30.5	29.8	30.0	29.9	30.0	29.9[5]
Nov. 19[4] G	26	250[5]	30.7	31.1	33.1	31.5	31.0	30.1	30.8	31.2	31.1	31.2
Dec. 17[4] G	26	250	31.5	32.1	33.0	32.5	31.9	30.8	30.4	30.4	30.3	31.2
Mar. 24 G	26	250	30.5	32.0	32.0	31.3	30.4	30.4	30.4	30.0	30.7	31.1
Sept. 23 G	26	250	30.2	32.4	32.5	32.1	31.5	29.9	30.5	31.5	32.0[3]	
		Average	30.5	31.7	32.4	31.6	31.0	30.4	30.4	30.5	30.7	30.7
Dec. 8 G	52	500	33.2	35.4	34.1	33.9	33.4	33.4	33.7	34.4[3]		
Dec. 22 G	52	500	31.5	35.9	35.4	34.7	33.3	34.1	33.4	33.1		
		Average	32.4	35.7	34.8	34.3	33.4	33.8	33.6	33.8		
Dec. 13 G	104	500	31.0	35.4	37.8	38.0	38.0	37.6	36.4	35.2	35.4[3]	

[1] U = Universal apparatus; G = Gasometer apparatus.
[2] Calculated to one half hour from five 10-minute periods.
[3] Calculated from results for less than a half hour (15 to 29 minutes).
[4] 1925.
[5] The water in the experiment of Nov. 19, 1925 was at 16.8°C.

hours, and two of the three also gave increases in the third and fourth half hours. These three experiments, together with the one on January 27 in the 15-gram group, suggest that the reaction to the ingestion of levulose is greater when the basal metabolism is high than when it is low. The average increase in the first half hour for the four experiments was 0.7 and in the second, 0.5 calorie. These increases are not so great as in the two preceding groups with 10 and 15.5 grams of levulose and the average decreases in the succeeding half hours, namely from 0.5 in the fifth half hour to 1.2 calories in the ninth half hour, were greater than in those groups. For the group as a whole, therefore, there was not so marked an increase in metabolism as for the preceding group.

In all six experiments with 26 grams there was an increase in the heat production in each of the first three half hours, and in five of the experiments there was an increase in the fourth half hour. There was a greater general increase in the heat production in the first four half hours, and a smaller change in the next five half hours than in any of the preceding groups. The net increases were of about the same order as those for 15.5 grams and were not so great as one would expect if the increases were proportionate to the amount of levulose ingested. With the exception of the 21-gram group, there was progression in the increases in heat production as the amount of levulose ingested was increased.

In both experiments with 52 grams, all succeeding half hours after the ingestion of the levulose showed an increase, the maximum being 4.4 calories in the first half hour on December 22. The total increase in 3½ hours, which was as long as the experiments were continued, was 12.7 calories. Both in 1 hour and in 2 hours the increase in heat production after 52 grams was nearly twice as much as it was in corresponding periods with one half the quantity of levulose. In three hours the increase was two and one-half times as great as it was with one half the quantity of levulose.

In the one experiment with 104 grams, there were increases in all of the periods measured and, in all likelihood, the total increase in heat production was not obtained, as the increase evidently continued longer than 4 hours. The maximum increase of 7 calories occurred both in the third and in the fourth half hour, and was equivalent to 22.6 per cent of the basal value. The increase in 1 hour was 11.2 calories, which is nearly twice the increase in 1 hour after 52 grams of levulose. In 2 hours it was 25.2 calories, which is nearly three times the increase after 52 grams; in 3 hours it was 37.2 calories, which is over three times the increase after 52 grams; and in 4 hours it was 45.8 calories, which is about ten times as much as the

increase in 4 hours with 26 grams, that is, one-quarter of the amount of levulose given in this experiment.

There was a gradual ascent in the increase in heat production above the basal values when quantities of levulose from 5.05 to 15.5 grams were taken. The increase after 26 grams was about the same as after 15.5 grams, but the increase after 21 grams was not so large as after 10 and 15.5 grams. The calculation of the true effect should include the differences in the negative changes in the periods following those in which increases were found. These negative changes were not so large for the 10, 15.5, and 26-gram groups as for the 5 and 21-gram groups. The summation of the average negative changes in the fifth to ninth half hours for the 5 and 21-gram groups was 3.7 and 4.4 calories, respectively, whereas in the 10, 15 and 26-gram groups it was 1.9 calories, 0.7 and +0.3 calorie, respectively. There was, therefore, in addition to the positive increases in heat production, a difference in the various groups of nearly 4 calories in the downward course of the heat production in the periods following those in which there was a rise in heat production over basal. The real elevation in heat production for the 10, 15 and 26-gram groups was greater by 4 calories than that in the 5 and 21-gram groups. The average increase in heat production for each group is shown in Figure 1, page 396.

Specific Dynamic Action

The increase in heat production due to 5.05 grams of levulose was on the average 0.5 calorie, which is equivalent to 3 per cent of the fuel value (heat of combustion) of the levulose; in the 10.1-gram series the increase of 1.8 calories equals 5 per cent. The summation of increases in the 15.5-gram group was 4.0 calories, which is equivalent to 7 per cent. In the 21-gram group, as pointed out in the discussion of the heat production, the increase was smaller in proportion to the amount ingested than in either the 10 or the 15.5-gram group, *i.e.*, only 1.5 calories, or 2 per cent. The summation of increases in the 26-gram group was 4.7 calories, which is 5 per cent of the energy value of the levulose ingested. In the discussion of the heat production, it was shown that to the positive increases above the basal value could be added about 4 calories which are due to the fact that the fall in the heat production from the fifth to the ninth half hour after 26 grams was not so large as that after 5 grams and intermediate quantities of levulose. Including this the amount of heat would raise the specific dynamic action for 26 grams of levulose to 9 per cent.

The increase in one of the experiments with 52 grams was 19.4 calories in 3½ hours, which equals 10 per cent, but the increase in the other experi-

ment was only 5.9 calories in 3½ hours, which equals 3 per cent. The average of the two would be 6.5 per cent of the fuel value. In neither experiment was the basal heat production reached after the ingestion of the levulose, so that the specific dynamic action is probably higher than the above values. The increase in the experiment with 104 grams was 45.8 calories in 4 hours, which is equivalent to 12 per cent, and this is minimum, as in the eighth half hour the heat production was still 4.4 calories above the basal value. Benedict and Carpenter (1918, p. 338) found, with 75 and 100 grams of levulose, 3 to 14 per cent, and Deuel (1927) has recently reported 7 per cent as the specific dynamic action of fructose. The specific dynamic action, then, rises for quantities of levulose varying from 5 to 15.5 grams, is low with 21 grams, and then rises somewhat more rapidly as larger amounts are given. There is more irregularity in the specific dynamic action of levulose than there is in the case of dextrose, but both sugars behave similarly in that the larger amounts result in greater increases of heat production on the percentage basis.

The Rôle of the Water Ingested with Levulose.

The rôle which the water plays in the changes in carbohydrate metabolized and heat produced, when sugars are given, has already been discussed in the previous contribution on the effect of dextrose and water upon these factors. If correction were made for the effect of water alone in the results for 15.5 grams of levulose, or under, the increases in both the carbohydrate utilized and heat produced would be augmented. With 21 grams of levulose (200 cc. of water), the change in carbohydrate would be increased, and the change in heat produced would be decreased. With 26 grams of levulose (250 cc. of water), the increases in carbohydrate, though diminished, would not be affected seriously, and the increase in heat production would be diminished by 2.6 calories. Correction for the effect of water, when the levulose (52 and 104 grams) was given in 500 cc. of water, would lower the increase in carbohydrate by about 3 grams and would lower the increase in heat production by about 7 calories.

The Significance of the Respiratory Quotient after Ingestion of Levulose.

One of the objects of this study was to determine whether the type of reaction observed after the ingestion of levulose is the same regardless of the amount given. A study of the respiratory quotients in these experiments shows that this is the case. There is a marked rise in the respiratory quotient to a peak, which takes place usually within 45 minutes. The main

difference in the results with the different quantities is the height to which the quotient rises and the duration of the period of the high quotients. If the basal respiratory quotient in each experiment is subtracted from the highest quotients for a single 10 to 15-minute period, the average rise in quotient for the different amounts of levulose is as follows: 5.05 grams, 0.05; 10.1 grams, 0.09; 15.5 grams, 0.18; 21 grams, 0.12; 26 grams, 0.16; 52 grams, 0.18; and 104 grams, 0.21. There is no marked difference in these increases when the amounts are above 10.1 grams, for 15.5 and 52 grams gave the same result. There is a tendency to longer periods of high respiratory quotients with increasing amounts ingested, the high quotients persisting for nearly two hours after 104 grams of levulose. Another fact of interest is that the rise in the quotient is independent of the basal respiratory quotient. In the 10.1-gram group, increases of 0.09 and 0.08 were found with basal respiratory quotients of 0.88 and 0.80; in the 15.5-gram group, a rise of 0.17 with basal quotients of 0.85 and 0.80; in the 21-gram group, increases of 0.13 and 0.12 with basal quotients of 0.83 and 0.87; and in the 26-gram group, a rise of 0.17 with basal quotients of 0.81 and 0.88, so that the reaction after levulose is uniformly independent of the original level of the respiratory quotient. High respiratory quotients after levulose, similar to those observed in the above experiments, have been found by nearly all previous investigators, most of whom have attributed the quotients to the process of formation of fat from levulose.

Joslin (1923, p. 233) was forced to the conclusion that the diabetic with respiratory quotient above unity converted levulose into fat. Dumont (1922) reported the beneficial effects of giving to diabetics those vegetables which produce levulose, and Root and Baker (1925) found increases in the respiratory quotient when levulose and Jerusalem artichokes were given to diabetic patients. Carpenter and Root (1928) demonstrated the absorption and utilization of the carbohydrates of the Jerusalem artichoke (presumably, for the most part, levulose and its polymers) in a diabetic patient with an absence of glycosuria, the prompt appearance of sugar in the urine when potato containing an equivalent amount of carbohydrate was given, and the equally prompt return to a sugar-free condition when the artichokes were again a part of the diet.

Campbell and Maltby (1928) have recently made a series of measurements of the carbon-dioxide combining power and lactic acid in the blood of patients to whom 100 grams of dihydroxyacetone, levulose, sucrose, glucose and maltose were given. They found that the ingestion of the first three substances resulted in a decrease in the carbon-dioxide combining power and an increase in the lactic acid of the blood, the greatest changes

occurring with dihydroxyacetone and the least with sucrose. On the other hand, they found little or no change after the ingestion of glucose or maltose. They conclude that the high respiratory quotients obtained with the first three materials were due to over ventilation and were not true metabolic respiratory quotients, whereas the quotients found after glucose and maltose were real indications of qualitative changes. However, it should be pointed out that compensatory decreases in the respiratory quotients are not found in the studies with levulose and sucrose. Other evidences in the literature of changes in the lactic acid in the blood when sugars were given conflict with the findings of Campbell and Maltby. Katayama (1929) found an increase in the lactic acid of the blood simultaneous with the hyperglycemia which took place after the ingestion of 1.75 grams of dextrose per kilogram of body weight in 22 normal and diseased subjects. Oppenheimer (1928) found no increase in the lactic acid of the blood either in normal persons or in patients with diseases of the liver when 50 grams of dextrose or levulose in 300 cc. of water were administered by mouth.

Douglas and Priestley (1924) have studied the changes taking place in the ventilation and the alveolar carbon dioxide in relation to the increase in the carbon dioxide elimination after the ingestion of 75 to 80 grams cane sugar. They found that the alveolar carbon dioxide remained practically unchanged throughout the periods of high respiratory quotients and that the percentage changes in the calculated alveolar ventilation were closely parallel to the percentage changes in the carbon dioxide elimination. They conclude that the respiratory center reacted to a uniform carbon dioxide level and that there was an adjustment of the ventilation to correspond to the changes in the carbon dioxide elimination. One is therefore justified in concluding that the high respiratory quotients which they obtained were true metabolic indices.

Part of the increase in oxygen absorption observed after levulose is ascribed by Campbell and Maltby to the increased work of over ventilation. Liljestrand (1918) found a maximum value of 0.7 cc. of oxygen per liter of increased ventilation, and the maximum increase in ventilation in any of the experiments in the series here reported was from an average of 5.7 liters per minute in the basal periods to 8.1 liters in the fourth 10-minute period of the experiment with 104 grams of levulose. If the above value for oxygen is applied (2.4×0.7), this increase in ventilation accounts for only 1.7 cc. of oxygen. The rise in oxygen consumption in this period was from a basal of 216 to 253 cc. per minute, an increase of 37 cc. Furthermore, neither the ventilation nor the oxygen fell materially until more than an hour and one half after the fourth period.

Campbell and Maltby comment that many of the high respiratory quotients after levulose are from isolated short periods and that one is not warranted in carrying out the calculations to a period of an hour from such short intervals of measurement. This comment applies neither to the investigations of Joslin nor to those here reported. Joslin's studies with diabetics were conducted with the patient in a respiration chamber (Benedict and Tompkins 1916) for successive half hour periods which usually totalled two hours, and our experiments were made in successive short periods with a total duration of more than two hours in the first series after the levulose was taken. In some of our experiments with the larger quantities, the high quotients were obtained for a period of an hour or more. In the experiment with 104 grams, the average non-protein respiratory quotient for 1 hour was 1.049 and for a half hour beginning 20 minutes later was 1.032. In view of this evident effect of levulose and of the fact that even small quantities gave large increases in quotients, it seems highly probable that the ingestion of levulose results in the formation of an oxygen-poor substance (fat) which may be either stored or subsequently burned.

The usual interpretation of respiratory quotients is that when they are over 1.00, formation of fat from carbohydrate takes place, but when they are below unity, the character of the metabolism may be either predominantly carbohydrate or predominantly fat, depending upon the level of the quotient. Benedict and Carpenter (1918, p. 248) have called attention to the possibility that formation of fat from carbohydrate may take place simultaneously with the utilization of the usual combination of protein, fat, and carbohydrate, even when the respiratory quotient is below unity. Cathcart and Markowitz (1927) have postulated that high respiratory quotients should be interpreted as indication of the formation of fat from carbohydrate and low quotients as an index of the opposite transformation. It is evident that if formation of fat from a particular carbohydrate takes place, it is an empirical interpretation of the respiratory quotient to conclude that this transformation occurs only when the respiratory quotient is above unity. Consequently, the probability of the formation of fat from carbohydrate, when the respiratory quotient is below unity, is not excluded.

Summary

The respiratory exchange of a human subject was determined while he was in the post-absorptive condition and during 3½ to 4½ hours after the ingestion of levulose in amounts from 5 to 104 grams, which were taken in water at 37°C.

The maximum respiratory quotient was found mostly during the second half hour after levulose, and, with the larger quantities, the increases in the quotients were larger and continued later than with the smaller quantities.

The increase in apparent catabolism of carbohydrate represented 30 to 34 per cent of the amount of levulose given the subject, with the exception of 14 per cent in the experiments with 5 grams of levulose and 53 per cent in the experiments with 15.5 grams. Although the values, 29 and 31 per cent, were found for 52 and 104 grams, not all possible increases are included as the experiments were too short in duration.

Increases in heat production were found in all groups, which varied from 0.5 calorie in 1 hour after 5 grams of levulose to 46 calories in 4 hours after 104 grams. The minimum increase in proportion to the amount given was 1.5 calories in 2 hours after 21 grams.

The specific dynamic action varied from 2 per cent with 21 grams of levulose to 12 per cent with 104 grams, although in the latter case the total heat increment was not measured.

Although the values obtained for carbohydrate after levulose are treated mathematically as increases in metabolism of carbohydrate, a discussion is given with respect to the significance of the changes in the respiratory quotient, and the probability is favored that the reaction after levulose is one of conversion of carbohydrate into an oxygen-poor substance (fat), even when the respiratory quotient does not rise above unity.

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EXPERIMENTAL NUTRITIONAL POLYNEURITIS IN THE RAT*

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THE question has frequently been raised in recent years as to whether the rat is a suitable animal for use in studies of the antineuritic vitamin, since the symptoms of the specific deficiency (polyneuritis) have not in the past been produced with regularity in rats, during the course of feeding experiments, and because of this, doubt has arisen as to the value of this technique. As recently as 1928 Kinnersley, Peters and Reader[1] make the statement that "it is not yet absolutely certain that rat tests can differentiate the curative factor in the vitamin B complex." Chick and Roscoe[2] rarely observed symptoms of polyneuritis in their animals on their basal diet K alone. Drummond[3] noted paralytic symptoms in three only out of a large number of rats on diets deprived of water-soluble vitamins. Opposed to these negative results, we have the earlier work of Schaumann[4] who found that rats confined to a diet of denatured horse flesh developed symptoms of paralysis. He states that three rats on this diet became lame after 25-28 days on this diet. All three animals survived in this condition for the eighty days of the experimental period. Hofmeister[5] was able to produce symptoms of polyneuritis in rats with considerable regularity. His work will be referred to again.

With the recent differentiation of the antineuritic vitamin from the more heat-stable substance (factor P-P, Vitamin B_2 or G) it becomes important to study the response of experimental animals to varying degrees of restriction of the vitamin, and to discover whether or not a characteristic train of symptoms may be produced in the rat, which may

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[1] Kinnersley, H. W., Peters, R. A., and Reader, V., Antineuritic Yeast Concentrates. III. The Curative Pigeon Test: A Critique. *Biochem. Jour.*, 1928, xxi, 276.

[2] Chick, H., and Roscoe, M. H., On the Composite Nature of the Water-Soluble B Vitamin. *Biochem. Jour.*, 1927, xxi, 698.

[3] Drummond, J. C., A Study of the Water Soluble Growth-Promoting Substance in Yeast I. *Biochem. Jour.*, 1917, xi, 255.

[4] Schaumann, H., Further Contribution to the Etiology of Beriberi. *Trans. Soc. Trop. Med. Hyg.*, 1911, v, 58. Cited by Hofmeister.[5]

[5] Hofmeister, F., The Beriberi of Rats. *Biochem. Zeitschr.*, 1922, cxxviii, 540.

be correlated with the severity of the deprivation of the vitamin in the diet.

The observations reported here were made in the course of experiments designed to measure the solubility of the antineuritic vitamin in alcohol 80 per cent by weight.[6] Over 100 animals were kept under controlled conditions for periods varying from a few weeks to several months. Their response, when graded additions of the antineuritic substance were made to the basal diet, has been carefully noted.

Experimental Methods

Healthy young rats of known nutritional history were placed at 28 to 29 days upon a diet believed to be quite free of the antineuritic vitamin but adequate, so far as present knowledge goes, in all other respects. The diet of Sherman and Spohn[7] for determination of vitamin B (using this term in the older sense of the word) was modified to include 15 per cent of dry bakers' yeast, autoclaved for three hours at 125°C. The autoclaved yeast replaced an equal weight of cornstarch and supplied the recently differentiated heat-stable factor, but, so far as feeding tests indicated, contained no important amount of the antineuritic vitamin.

Animals received this basal diet with or without the quantitatively graded additions of materials whose antineuritic potency was to be tested. These materials were dry bakers' yeast, alcoholic extracts of yeast, the residue left after this alcoholic extraction, and an alcoholic extract of ground whole wheat. In the preparation of the alcoholic extract and the extracted residue of yeast,[6,8] 400 grams of air-dry bakers' yeast were treated with 1500 cc. of alcohol (80% by weight), thoroughly stirred and allowed to stand at room temperature (20° to 25°C.) for 24 hours; then filtered with suction, and the yeast washed on a Buchner filter with 750 cc. of alcohol of the same strength; then again stirred with 1500 cc. of the alcohol, allowed to stand 24 hours, filtered and washed as before. The residue was dried in the air at room temperature. The extract obtained by combining the two filtrates and the washings was a clear yellow solution of p_H 6.1. It was concentrated on the steam bath and evaporated at

[6] Sandels, M. R., Dissertation, Columbia University, 1928.
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[7] Sherman, H. C., and Spohn, A., A Critical Investigation and an Application of the Rat Growth Method for the study of Vitamin B. *Jour. Amer. Chem. Soc.*, 1923, XLV, 2719.

[8] Sherman, H. C., and Sandels, M. R., Experiments with reference to the more heat-stable factor in the vitamin B group (Factor P-P, Vitamin B_2 or G). *Pro. Soc. Exper. Biol. Med.*, 1929, XXVI, 536

room temperature on to cornstarch. The dried product was ground and enough cornstarch was added to make the final weight of the preparation 200 grams.

Symptoms of Polyneuritis in the Rat

Animals on the basal diet alone grew somewhat during the first 7 to 14 days then declined rapidly and died within 25 to 40 days' time, the average survival period being 38.1 days. The majority of these drooped in their cages and became weak and unsteady on their feet, but rarely showed characteristic symptoms of polyneuritis. At death cyanosis was usually prominent.

Animals receiving the vitamin in amounts which were measurable, but which were insufficient for protection, developed almost without exception, typical symptoms of polyneuritis. Head retraction was usually one of the earliest symptoms. In its mildest form it appeared as a nervous jerking of the head when the animal became excited. In more aggravated cases, the opisthotonic position of the head was quite marked. There was sometimes complete paralysis of the hind legs, but more frequently the nervous involvement manifested itself in a spastic gait, or in loss of muscular control, especially when handled. Animals were apt to hold their heads on one side and walk in circles. The survival period appeared to be correlated with the amount of the vitamin supplied.

An amount of the vitamin which allowed approximately net maintenance of the weight of the experimental animal over an eight week period, was frequently insufficient for protection from the deficiency. At maintenance level a subacute or chronic type of polyneuritis often developed. Animals in this condition usually maintained life for several weeks, and the majority of these showed definite symptoms of polyneuritis continuously after the deficiency had once become manifest.

A few typical protocols of animals showing characteristic behavior are given below:

1. Protocols of litter mates, showing graduation of symptoms when fed graded amounts of antineuritic vitamin. The basal diet used in these experiments, a modification of the Diet 107 of Sherman and Spohn had the following composition:

Casein, extracted with 60% alcohol	18%
Salt mixture (Osborne and Mendel)	4%
Cod liver oil	3%
Butter fat, filtered	7%
Cornstarch	53%
Autoclaved bakers' yeast (3 hrs. at 125°C)15%	15%

Rat B2406. Put at 28 days of age upon the basal ration designed to be devoid of antineuritic vitamin, without further addition. During the first week it gained somewhat, then declined rapidly and died without noticeable symptoms of polyneuritis, on the seventeenth day of the experimental period.

Rat B2403. Put at the same age upon the same basal ration but received six times weekly, as supplement, the alcoholic (85%) extract from 0.400 gram whole wheat. During the third week the animal developed acute symptoms of polyneuritis, the hind legs being completely paralyzed. It died within a few days.

Rat B2402. Put at 28 days upon the same basal ration with supplement six times weekly of the alcoholic (85%) extract from 0.800 gram whole wheat. The animal grew considerably during the first two weeks, then lost slightly. During the fourth week polyneuritis developed and coincidently there was a sharp loss in weight. Following this there was a slow gain in weight, although pronounced symptoms of polyneuritis were present continuously and included head retraction, spastic gait and marked excitation. This latter manifested itself in the animal's frantic circling of the cage whenever there was movement or noise in the laboratory. Four weeks later (the end of the eighth week of the experiment) the animal died in convulsions.

II. Protocols of animals showing typical subacute or chronic polyneuritis.

Rat L129. 12/3/27 Put at 28 days of age on the same basal diet, designed as devoid of antineuritic vitamin, plus 0.200 gram yeast six times weekly. 1/4/28 Head retracted. 1/9/28 Spastic gait, head retracted. 1/13/28 Cartwheel turning, drags hind leg. 2/3/28 For past five weeks animal has showed continuously the classic symptoms of polyneuritis, spastic gait, head retraction and convulsive seizures when handled. Chloroformed.

Rat 23065. 12/12/27 Put on the same basal ration plus the extract from 0.400 gram yeast three times weekly. 1/19/28 Polyneuritic. 1/30/28 Very weak and sick, partly paralyzed. 2/6/28 Condition improved, more lively, better control of movements. 2/13/28 Paralytic seizures when handled. Chloroformed

Discussion and Conclusion

This subacute or chronic type of polyneuritis has seemed of particular interest. Vedder and Clark[9] in 1912 described a similar condition in fowls and their findings were later confirmed by Weill and Mouriquand.[10] Recently Randoin and Lecoq[11] reported a similar condition in a pigeon receiving an amount of vitamin insufficient for full protection. Hofmeister[3] described this subacute type of polyneuritis, and, as in the present study, found the condition associated with shortage rather than complete absence of the antineuritic vitamin. The present work confirms that of Hofmeister and extends it somewhat in showing a definite gradation of

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symptoms correlated with the extent of the deficiency of the vitamin in the food supplied.

It seems probable that the irregular appearance of symptoms in earlier experiments was due to differences in the basal diets used and to the failure to recognize the need for inclusion of the heat-stable vitamin G. Results secured in the present study leave no reasonable room for doubt that the rat is sensitive to the specific deficiency and will develop well marked symptoms of polyneuritis, with sufficient regularity to be a valuable aid in determining the extent of the deficiency in antineuritic vitamin to which the animal is subjected.

FURTHER EXPERIMENTS ON THE INFLUENCE OF FOOD UPON LONGEVITY*

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THE available data regarding noteworthy cases of longevity in man have given the impression that this depends almost entirely upon inheritance. The limitations of the method of investigation in such cases, however, may quite conceivably have tended to obscure or minimize the importance of food, and perhaps of other factors. In compiling the available facts regarding a person who has attained to great age, the ages reached by parents and grandparents will usually stand out as clear-cut quantitative data; while it will be quite impossible to obtain equally clear-cut and quantitative data as to the food consumed throughout a life-time.

This latter factor can, however, be studied experimentally by the use of such a laboratory animal as the rat; and the suitability of this species for use in studies of nutritional problems of human importance has been fully discussed by Osborne and Mendel (1), while further evidence is afforded by the work of Folin and Morris (2) which showed close similarity of the end products of metabolism in the two species.

McCollum and others (3) have repeatedly shown that the normal length of life of experimental animals (rats) may be shortened in almost any desired degree by dietary deficiencies of varying degrees of severity.

Our own problem has been to determine whether, starting with a food supply and nutritional condition already adequate and normal, it is possible by improvement of the food to induce a definite increase in longevity.

In the present experiments the original diet (Diet A) consisted of a mixture of one-sixth dried whole milk and five-sixths ground whole wheat, with table salt and distilled water. Families of experimental rats are still thriving after 21 successive generations on this diet. This is probably an unprecedentedly rigorous test of the adequacy of a food supply and shows beyond question that this original diet was certainly adequate.

Simultaneously, parallel lots of animals, of the same heredity, have been fed a diet differing only in that the proportion of milk powder in the food mixture was increased to one-third (Diet B).

While Diet A was clearly adequate, Diet B proved to be better, and

* Published as Contribution No. 618 from the Department of Chemistry, Columbia University.

induced, in addition to other evidences of improved nutrition (4), *both* a decrease in infant mortality *and* an increase of ten per cent in the average length of life of the adults.

The two groups of animals, which as already mentioned were of the same heredity, were kept under identical conditions in all respects except for the difference in food. The higher proportion of milk in Diet B means chiefly, in chemical terms, a richer intake of calcium, of vitamins A and G, and of certain of the amino acids. Experiments designed to determine to which of these chemical factors the increase of longevity is attributable are now in progress but not yet sufficiently advanced to permit of final conclusions. From the results thus far obtained it seems probable that the increased intakes of calcium, of vitamin A, and of vitamin G may all have contributed to the higher degree of health and the increased length of life.

That the increase of longevity was real and not accidental is made clear both by simple statistical treatment of the data and also by grouping them in such manner as to afford a series of comparisons (of the animals on the two diets) in terms of the percentages attaining to different degrees of longevity. The evidence as assembled in these two ways is briefly summarized in Tables I and II, respectively.

A part of this evidence was presented in a preliminary report of about a year ago (5). Since that time the numbers of animals compared have been considerably increased and the findings have become more conclusive.

TABLE I
COMPARISON OF LONGEVITY OF RATS ON DIETS A AND B

	Diet A		Diet B		Difference of Length of Life in Days
	Number of cases	Average Length of life in days	Number of cases	Average Length of life in days	
Males	135	571±8.0	124	635±8.5	64±11.7
Females	196	603±8.0	163	669±7.8	66±11.2

The average lengths of life and the differences of these averages, as given in Table I, are followed by estimates of their probable errors computed in the usual manner. While it is true that such computation is based upon an assumption of symmetrical frequency distribution and therefore may or may not be precisely accurate for data of this particular kind, yet, inasmuch as these data belong to the category of natural phenomena to which, according to Reitz and Mitchell (6) the usual calculations of probability may normally be expected to be applicable, there is good reason

to believe that the quantitative relations thus computed are at least approximately correct. This being the case, and the increase of longevity being 5.6 and 5.9 times its probable error, for males and females respectively, it follows that, speaking mathematically and from the standpoint of this mode of interpretation alone, the chances are about ten thousand to one (10,000:1) that the increase in longevity here found is a true difference due to the food, and not an accidental difference nor due to unknown causes.

The findings are established with still greater certainty when our basis of scientific judgment is broadened to include a further consideration of the data from the point of view summarized in Table II. This makes possible a series of nine comparisons, and in every one of these the greater longevity attained upon Diet B than upon Diet A is clearly apparent.

TABLE II
INFLUENCE OF FOOD UPON ATTAINMENT OF DEFINITE AGES

Percentage of Lives Longer than:	Males		Females	
	On Diet A	On Diet B	On Diet A	On Diet B
600 days	42.9	65.3	54.1	73.0
700 days	14.8	32.3	27.6	43.6
800 days	2.9	10.5	12.2	15.9
900 days	0.0	1.6	2.6	5.5
1000 days	—	—	0.5	1.2

Hence it may be regarded as established beyond any reasonable doubt that, starting with a diet which is already clearly adequate, it may still be possible to induce a very significant improvement in longevity by enriching the diet in certain of its chemical factors.

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Editorial Review*

BIOLOGICAL VALUES AND THE BEHAVIOR OF FOOD AND TISSUE PROTEINS[1]

I ONCE read, somewhere, of two wanderers, one a German, who came to a fork in their road. To the left the arrow read, "To Paradise"; to the right, "Problems on Paradise." The German, naturally, chose the latter.

Consistently following this national characteristic of the Germans, I must not be expected to give a detailed summary of the biological values of all possible pure proteins, or of the synthetic and naturally occuring protein mixtures; or of the practical results which have been achieved through the knowledge of these facts in the nourishment of sick and healthy persons, in the breeding of domestic animals, and in the production of milk, meat, eggs, etc.

I. Biological Value of Food Protein

I would rather discuss the methods employed in the determination of biological values and stress particularly the individual factors which influence the practical value of a protein. In this connection, I wish to criticize the methods. The one method which uses the nitrogen equilibrium(balance) experiment, I should like to call the analytical method. By means of this method determinations on human beings can be carried out. The other makes use of an artificial diet consisting of pure nutritive elements, with which small experimental animals are kept alive or in a growing condition. I should like to call this latter method the synthetic one.

The synthetic method gives us a definite answer to the question whether the protein is useful or not and permits an approximate measure of the

* The writer, Professor Karl Thomas, was for many years associated with Professor Max Rubner in the Hygienic Laboratory and later in the Physiological Institute at the University of Berlin. He was for three years Assistant in Chemistry at Tübingen and made his habilitation for physiology at Greifswald with Bleibtreu in 1912. He returned in 1913 to Berlin where he was in charge of the Kaiser Wilhelm Laboratory for Arbeits-Physiologie under the general direction of Prof. Rubner. Since 1921 Dr. Thomas has been Professor of Physiological Chemistry at the University of Leipzig, sharing the chair originally occupied by C. Ludwig. While in Rubner's laboratory Dr. Thomas introduced a method for establishing the biological value of different proteins. With himself as subject, he studied the effects on nitrogen excretion of ingesting different protein foods, in comparison with a diet containing no protein. From these results he was able to give numerical values to different kinds of protein. This conception of biological value depending on composition has become thoroughly established in the physiological literature. Prof. Thomas also has contributed important researches in intermediary metabolism, such as the significance of creatin and the beta-oxidation of ω-amino fatty acids. He has sought in many studies to make the methods of synthetic organic chemistry serviceable to the physiology of metabolism. With Knoop of Tübingen he is now editor of the well-known Hoppe-Seyler's Zeitschrift für Physiologische Chemie.—Ed.

[1] Revised from an address delivered at the University of Rochester Medical School, September 30, 1929.

usefulness through a comparison of the amounts of various proteins necessary for maintenance or growth.

With the nitrogen balance method, on the other hand, the research worker obtains more nearly quantitative results, since its basis is the definite bodily requirement which Rubner has called the wear and tear factor.

What is it that is designated as the practical or biological value? I can explain it perhaps most simply by means of the figure on page 421. I plot the grams of nitrogen which express the bodily requirement on the ordinate O-C; on the abscissa I plot the diet nitrogen. Through C a line is drawn parallel to the abscissa on which I plot the nitrogen excreted, E, E_1, E_2. When we have nitrogen equilibrium the connecting line between the end points of intake and excretion E-I is at right angles to the abscissa and parallel to the ordinate. When the balance is positive, then this connecting line E_1-I leans to the left, toward the ordinate; when it is negative, E_2-I leans to the right, away from the ordinate.

The least nitrogen with which I can achieve a balance just manages to cover the nitrogen requirement; $100 \times \tan \alpha$ expresses the relation in which the diet nitrogen can replace the requirement; in other words, the biological value. If the food protein is exactly equal to the body protein destroyed in katabolism then we have O-I = C-E = O-C

$$\alpha = 45° \text{ and}$$
$$100 \times \tan \alpha = 100$$

Let us study this picture still further. The distance O-C represents the need of N on a diet which consists mainly of carbohydrates and contains no protein. The entire requirement must then be cared for by the body's own nitrogen; the protein catabolism under these circumstances being the lowest possible. Although I am not convinced that the N excretion actually represents the protein metabolism under the circumstances of the nitrogen minimum, nevertheless we judge the protein requirements by the N excretion in the usual manner.

Is the distance O-C really constant? It consists of three components: The urine N (u) the feces N (f) and the N which leaves the body by means of the skin. The last of these cannot always be neglected. When, for instance, in the case of heavy physical labor, the sweat glands are more active, it is possible for the sweat to contain 0.2 gm. more N per hour than when the body is at rest (F. G. Benedict, 1906). One must bear in mind, however, that this amount rises and falls only in direct proportion to the rise and fall of the N metabolism in the entire body. At the level of the

N minimum it may be neglected, since it amounts only to 0.13 gm. per day (Thomas, 1910).

The feces N (f) is present not only on the ordinate as part of the N requirement, but on the line parallel to the abscissa as part of the N excretion. It consists of two parts; 1st, the exogenous portion, derived from the protein intake, and 2nd, the endogenous portion derived from the secretions of the gastro-intestinal glands. Both are variables, and are dependent upon, 1st, the kind of food protein, and 2nd, on the character and quantity of the ingested food as a whole. In the N minimum experiments, which I conducted, the ingested N was kept at an approximately constant

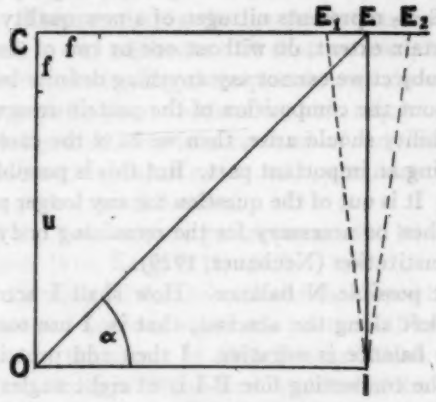


FIGURE 1.

quantity. In addition to this precaution, the foodstuffs that were absorbed with difficulty could be avoided.

The excretion C-E must, naturally, be entirely free of N-containing residues from previous protein-rich diets. These must be removed by a sufficiently long preliminary period. To this end a week at most sufficed, as I proved on myself, and as was later shown by Martin and Robison (1922) in detailed observations. Only in the case of very great overfeeding of protein does the return of excretion to the wear and tear quota require a longer time (as much as 2–3 weeks) (Thomas, 1910). This is probably due to the fact that under the circumstances not only is a removal of N residues involved, but also a complete destruction of protein reserves. That which finally remains in the tissue as extractive N is not residue but, according to Mitchell, Nevens and Kendall (1922) a definite component of the organism.

Thus far we have been considering only the katabolism of body protein in the case of protein starvation, in other words, the nitrogen requirement. We, have, after a fashion, concerned ourselves only with the ordinate. Let us now turn to the abscissa. In any experiment to determine the biological value, the body requirement should be just balanced by the food protein. I must, therefore, achieve a minimum under the following conditions:

1. Nitrogen equilibrium, or balance ± 0; that is, when I-E is at right angles to the abscissa. This is important, for in the case of a negative balance when the body contributes protein of its own, it is possible that that protein will be the one most necessary and least present unit—in other words, the line $E-E_2$ represents nitrogen of a new quality! The body can, perhaps, to a certain extent, do without one or two of the important proteins. On that subject we cannot say anything definite because we do not know enough about the composition of the protein reserve. If the above mentioned possibility should arise, then we have the case of a less important protein taking an important part. But this is possible only for a limited, short time. It is out of the question for any longer period simply because it would then be necessary for the remaining body protein to take on a different constitution (Neubauer, 1929).

2. The lowest possible N balance. How shall I accomplish this? I move from the left along the abscissa, that is, I use too little food protein so that the balance is negative. I then add protein daily in small amounts until the connecting line E-I is at right angles to the abscissa. This method is better than the one involving a start with a larger amount and daily decreasing the intake. The danger of protein residues remaining is too great. How is it in the case of such incomplete proteins as gelatine? I intentionally did not study them. In that case O-I would be ∞ and tangent α then $= 0$. Robison (1922) later confirmed this theory on himself.

3. The length of the distance O-E depends also upon the speed of absorption. The proteins are not suddenly and completely split into free amino acids in the gastro-intestinal tract. This takes place in steps as has been shown by artificial ferment hydrolyses. Therefore, every moment sees a different mixture of amino acids absorbed, or, in other words, a protein of a different biological value. This, however, is minimized by the action of the pylorus which permits the passage of food only in small portions. Thus, as the most difficultly split acids are set free, and absorbed only in the lowest portion of the ileum, we have at the same time an absorption of the more easily set free acids, from a portion passing later through the pylorus, in the duodenum and upper jejunum. All of the

amino acids, which are absorbed at the same time, meet again in the portal vein. The proportions of the amino acids to each other are not changed by the slow absorption of some of them, except perhaps slightly at the beginning and at the end of the process. The amino acids in the diet should take care of the body's requirements for a period of 24 hours! The question is whether this requirement shows a regular daily variation. Perhaps only a deviation for definite amino acids? The possibility is logical; we know nothing of the facts and are at present in possession of no instrumentality which will make the point clearer.

Do the absorbed amino acids cover the need of the last few hours of the day? Ordinarily yes, simply because we invariably consume more protein than we require. The question is whether this is also the case in the small amounts involved in our minimum. Of that we are not certain. I have therefore distributed the feeding of the protein over the entire day. In the case of many, especially plant foodstuffs which require a longer time for mastication and digestion, this is taken care of automatically; but with such rapidly absorbed foods as meat, milk, etc., one must attain this advantage through fractional feeding.

The ability of the liver to store protein must also be considered in this connection (Grund, 1910; Berg, 1922, 1926).

We have now discussed all the theoretical difficulties which lie in the way of the determinaton of the food value of a protein. I can, perhaps, summarize them best in the words of Miss Jordan Lloyd, a student of Sir Gowland Hopkins. She says "The idea of expressing the value of different food proteins on an arithmetical scale is theoretically sound, but the practical method by means of which these values are to be obtained have been the subject of much discussion." I will only briefly present the following extracts from my results.

The proteins in potatoes fulfill our needs better than those in bread; finest wheat flour contains a less important protein. In contrast, milk presents a very important product, not only to fulfill the requirements of addition, but also those of turn-over. That need not astonish us, when we recollect that in the case of the rapidly growing calf the turn-over or the wear and tear quota comprises an appreciable portion, more than half, of the entire required protein. Milk is also probably better adapted to the needs of the grown-up than meat. Muscle meat represents only a single organ adapted to a definite function and requiring specific protein material; the wear and tear quota represents, however, the common wastes of all the organs of the body. Later workers have confirmed these results.

I believe I have now covered everything on the N balance method for

the determination of the biological value of the proteins, at least everything that can be called a definite result. We have in addition criticized and, I trust, objectively, the method in all details. I should now, however like to call attention to two additional points.

First, to the fact that comparatively few studies have been made where this method has been used. Very often, and especially here in this country, proteins have been examined only in rat-feeding experiments or, in other words, by the synthetic method, as I called it, in contrast to the analytical or balance method. Why is this the case? Martin and Robison write that the N balance type of experiment seems simple, but is not so in fact! They are right indeed! Accurate N minimum experiments can rarely be carried on for a sufficiently long period. It is possible with bread, potatoes, rice, corn and similar food stuffs, which are naturally of high starch content, but not with a diet which consists mainly of the unappetizing pure starch, milk sugar and cane sugar. I am more firmly convinced to-day, than twenty years ago, that the N-free control diet is necessary but that we can supplant the sugar to a great degree by pure fat and thus achieve a more tasty and varied diet (Zeller, 1914; Murlin, 1907).

The synthetic method presents the advantage that experiments can easily be carried out for any length of time and that the proteins can be tested on growing as well as grown animals and in addition on a large number of animals. The results give therefore good average values. Still another advantage is the fact that the long-continued experiments, which often require a great deal of expensive pure proteins can be carried out by the use of small experimental animals. The disadvantage lies in the fact that in lengthy experiments vitamin preparations must be administered which are not entirely free from protein or amino acids.

Secondly, I should like to point out that I have not myself carried these experiments further—I do not know whether anyone has noticed it; I never found any indication to that effect in the literature! This, however, seems to me to be the proper time to explain myself. The nitrogen balance method has a purpose when one desires an arithmetical expression for the biological value of a protein; the synthetic method yields merely approximate comparative values. But the arithmetical value can very easily vary by the addition of small amounts of any of the rarer proteins; or, differently expressed, the value changes noticeably when a less important protein is completed by the addition of some other one. In the meaning of "completion" we can already see the possibility of two very unimportant and valueless proteins combining to form a mixture of extremely

high value. That was clearly shown through the beautiful experiments of Osborne and Mendel (1915). Gliadin alone does not produce growth in young rats but can keep them at constant weight. Gelatine cannot even produce these results. The two together, however, guarantee excellent growth. All that was necessary was the enriching of the almost lysin-free gliadin with the relatively lysin-rich gelatine. The enriching of white wheat flour with powdered milk, as performed by Sherman (1924) is another example. There are many similar combinations. It should be mentioned that the natural foodstuffs never contain only one single protein. In milk, casein is enriched by lactalbumin; in bread prolamine by glutenine. You can see, therefore, that while the physiologist might be interested in determining the biological value of any single pure protein, the hygienist, who must deal with the practical side of the question, must bear in mind that the value cannot be obtained from the sum of the values of the individual components of the diet, but rather, the value must be experimentally determined for each different diet. The human, moreover, changes the nature of his diet from day to day. Average values for him are out of the question. It is quite another thing in the feeding problems for domestic animals. In this case such experiments have a real practical value and have been thoroughly studied here in the United States from the viewpoint of national consumption and national development.

I should like to compare the numbers indicating the biological values of proteins with the numbers well known as the fat constants. Both numbers are of practical value; they help in scientific research work, but they are not a part of it.

What we need to know is:

1. Which amino acids must be present in the food,
2. How much we require of each,
3. And to what purpose.

These questions deal with the carbon skeleton of the amino acids, and not with their N content. The N is the least important of the elements composing protein. Because of the ubiquity of the Kjeldahl method and the lack of methods to determine the different carbon skeletons, we deal with the N, but we should not do so. Armed in the future with knowledge of the behavior of the carbon skeletons we shall be able to calculate the value of every protein mixture as it occurs in a diet. The duty of the scientist is to furnish the fundamentals to the practitioner, and to make certain of these fundamentals is in particular the duty of the physiologist.

II. Behavior of Food and Tissue Protein in Metabolism

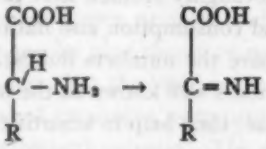
The idea of "food protein" must apparently surrender to the name "amino acid mixture". Does the same apply to the idea of "tissue protein", that is to say, are the katabolic processes the same in both cases? I am using the term tissue protein to cover the protein of the living substance of all tissues. This excludes all of the "dead", enclosed, reserve material of the cells as, for instance, all of the extractives and excretions and intermediary products between the cell walls.

As food protein we must include all those amino acids which do not serve for replacement in the wear and tear factor or, in other words, that portion which we labeled as superfluous.

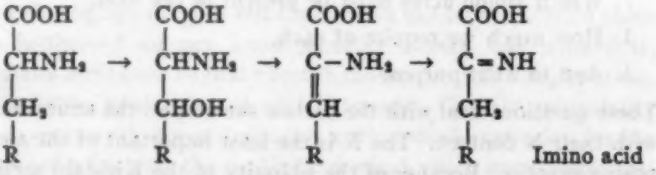
These amino acids are deaminized. The N-free carbon chains are either further synthesized or stored as sugar or, under special circumstances, are excreted as acetone or completely oxidized.

The deamination belongs to the first processes in katabolism and takes place in the liver (Mann and Magath, 1924).

Let us follow the process of deamination in single steps. It proceeds either by means of oxidation or reduction, as has been shown by Knoop, Dakin, Neubauer, Embden and Wieland. We can write the formulae as follows:



According to Dakin (1926), however, we can also write the reaction thus:



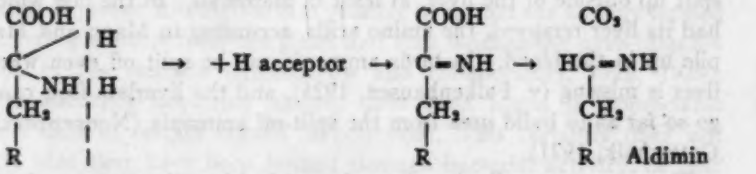
At this point one must recollect, however, that α-amino, β-hydroxy acids have never been isolated as free acids but always as a component of protein. Be that as it may, the important fact is that the imino acid is an intermediate product in both cases.

What is the next step? Does the deamination by hydrolysis come first and is then followed by the loss of carbonic acid, or do the steps take

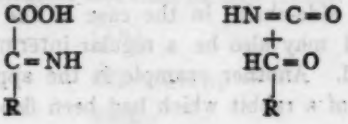
place in the reverse order? Or perhaps, as a third possibility, simultaneously?

Until a few years ago, it was believed that only the alpha keto acids were the first intermediate products, for these acids probably often resulted in the katabolism brought about by yeast. In normal as well as abnormal conditions of metabolism of animals and humans we have a complete parallelism between them and the respective amino acids, and this is true moreover of foreign as well as of native amino acids. By the feeding and blood perfusion of foreign amino acids it has been possible to isolate, and very often in large amounts, the respective keto acids. In spite of this it is my opinion that these are only probable proofs and that one must be extremely careful in the biological sciences in drawing analogies from such results. For perhaps the keto acid can only be isolated, or possibly occurs only, when the work is carried out under unnatural conditions with a carbon skeleton foreign to the body and with a surviving, that is to say, a dying organ, or by flooding of the animal body or the yeast cell with the amino acid under conditions which would never occur naturally.

I should, therefore, rather draw the conclusion that only under these unnatural conditions does deamination precede decarboxylation. We can draw no conclusions as to the normal procedure from these experiments. Wieland and Bergel (1924) oxidized the amino acid catalytically with pulverized palladium and animal charcoal. Under these circumstances decarboxylation preceded deamination, so that aldimin occurred as an intermediary product. They bring up the question whether the katabolism in the animal body does not also take the same course, but they feel it is not to be answered by analogous experiments with biological materials.



It is also possible for deamination and decarboxylation to occur simultaneously after the manner of the splitting off of cyanic acid.



These deductions help renew the old hypothesis of F. Hofmeister concerning the oxidative urea formation. Fearon (1926) supplied the new equations using the work of E. A. Werner on the constitution of urea as a basis. According to this theory the carbamid form $O=C\begin{smallmatrix}NH_2\\NH_2\end{smallmatrix}$ does not exist, but instead a cyclic acid $HN=C\begin{smallmatrix}NH_2\\|\\O\end{smallmatrix}$ in which the cyanic acid is already formed. Thus we apparently must return to the 100-year old Wöhler urea synthesis; Hoppe-Seyler and Salkowski permitted it to stand as re gards the animal body. Frankly, cyanic acid has never been definitely proved to be present in the blood, opposing Montgomery's view (1925), and what is more, it cannot be so long as the methods in use remain as incomplete as they are to-day.

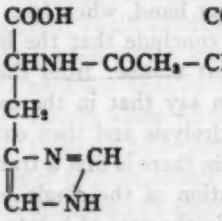
To sum up; the main process involved in the catabolism of the *superfluous*, ingested amino acids is the building of fatty acids with one less carbon. Oxidation, deamination and decarboxylation are three steps of a chain reaction. In my opinion, the shortening of the carbon skeleton right at the beginning is of great biological importance. Only this process renders it impossible for the carbon residue to be used in the synthetic preparation of amino acids. The amino acid is thus completely removed from the intermediary protein metabolism.

The nitrogen, to further develop these ideas, plays a special part because it is the presence of the amino group which diverts the entire amino acid to that place where it may be used. That is to say, to the point at which a deficiency has arisen through the wear and tear of the tissue protein and, moreover, where the deficiency can be corrected only by that one amino acid. The amino group, therefore, protects the entire acid from being split up outside of the liver, at least in mammals. In the dog which has had its liver removed, the amino acids, according to Mann and Magath, pile up in the blood. In birds ammonia can be split off even when the liver is missing (v. Falkenhausen, 1925), and the liverless frog can even go so far as to build urea from the split-off ammonia (Nonnenbruch and Gottschalk, 1921).

There are, naturally, in connection with this main process, other processes. This is perhaps shown by homogentisic acid formed from the oxidation of the benzene nucleus of tyrosin before or together with the shortening of the side-chain, in the case of the alcaptonuric patient. Homogentisic acid may also be a regular intermediate product in the healthy individual. Another example is the appearance of kynurenic acid in the urine of a rabbit which had been flooded with tryptophane

(Matsuoka and Yoshimatsu, 1925). Also tyrosinase oxidizes free tyrosine to melanin (Raper, 1926, 1927). Thus we see that for the most part the superfluous, free amino acids are shortened and deaminized and their carbon skeleton remainder is utilized.

We turn now to a consideration of the metabolism of tissue protein. The protein amines are formed by means of decarboxylation. The carnosin content of the muscles is not affected by the protein content of the food. Carnosin is present in different, but always constant amounts, depending upon the species and kind of muscles (Clifford and Mottram, 1923). Thus, in order that carnosin may be formed directly from food protein, histidyl aspartic acid must be preformed and this must be absorbed in the form of an asparagyl-histidin, a supposition which is highy improbable.

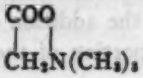


Formation of Carnosin

We do not know how creatinine is formed, but we do know that it is not formed from free arginine, even when the arginine is administered subcutaneously, thus avoiding the arginase-rich liver and reaching directly the arginase-free muscles. The small increases in creatinine excretion which Thompson (1917) claims to have obtained are not very convincing.

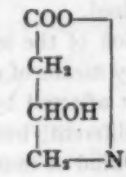
Epinephrin has never been obtained by the addition of tyrosin to a suprarenal press juice, nor even by the perfusion of the glands themselves. By means of proper stimulation it is possible to extract the already formed and stored epinephrin from the gland and thus weaken the pyro-catechin reaction of the medulla (Anitschkow and collaborators, 1928, 1929).

Other naturally occurring amines have been found in appreciable amounts only in certain organs (hypophysis). This certainly does not indicate that they have been formed through bacterial activity, or that they are merely accidental components of these organs. The fact that the amounts are so small, might be explained by the ease with which they can be oxidized to fatty acids and then further broken down. The betaïn



Betaïn

content of the common beet did not increase, no matter how much glycin was introduced (Ackerman, 1913). γ-butyric-betain is regularly found in the urine in the case of phosphorus poisoning as is also carnitine,



Carnitine

the betaïn of the γ-amino-β hydroxy butyric acid. The mother substance of both of these may be glutamic acid or proline and its respective oxy-acid. Both amino acids on the other hand, when free, are converted to urea.

From such observations I conclude that the breaking down of tissue protein proceeds in a different manner from that of the food protein. Compactly expressed, we can say that in the case of the food protein there is first digestion or hydrolysis and then oxidation or assimilation; in the case of the tissue protein there is first a transformation and limited oxidation and then a separation of the single building stone from the complex protein molecule as in the case of betaïn, or of a dipeptid in the case of carnosin, or of a tripeptid in the case of glutathione. An idea of Hofmeister's is that the peptid is to a certain extent a glycin chain and that the organism may obtain such extraordinary amounts of glycin as are found, because the side chains are oxidized away and the remaining glycin chain breaks up.

I have more precisely characterized the idea which Folin evolved, namely, that the exogenous portion of the protein metabolism results in other residues in the urine than the endogenous portion.

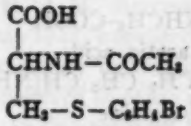
As further experimental support of this idea, I have requisitioned cystin. According to Folin the neutral sulphur in the urine is an end product of the endogenous metabolism. Cystin furnished in the diet is oxidized to sulphuric acid, even under the conditions of the nitrogen minimum. In the case of the cystenuric patient it is possible even under these conditions to isolate small amounts of cystin in the urine. Often these traces disappear. They rise and fall with the increase or decrease of the ingested amounts but do not parallel them. The cystin disappears from the urine only, however, on the addition of large doses of sodium bicarbonate, and therefore that portion of the neutral sulphur which is not free cystin, is increased.

Cystin is set free in the case of a healthy person. For the hair continues to grow under the conditions of a protein-free diet and contains, as we may freely assume, according to Abderhalden, its normal cystin content. An interesting and suggestive observation on molting hens has recently been reported by Ackerson, Blish and Mussehl (1926). On a N-free diet molting hens break down tissue protein in order to build up the new feathers. Small amounts of cystin prevented this breakdown to a certain extent.

The cystin metabolism of the liver also continues unchanged. In work of our laboratory, not yet published, Mr. Mukoyama found taurocholic acid unchanged in N starvation experiments covering a long period of time and thus confirmed the results of Whipple. Mukoyama was even able to increase the taurine content of the bile with the aid of cholic acid (very much at the beginning and then less so) and after exhausting in this way the amount of cystin stored, could produce taurocholic acid formation from taurine and cholic acid fed at the level of the N-minimum. From Bergmann's experiments we knew that cystin was the mother substance of taurin but he did not clearly show whether free cystin could be employed for this purpose. It might have been possible that it had at first become a part of the liver protein before it could be drafted for this purpose. We do not need Mukoyama's experiment to convince us of these results. Taurocholic acid may be, therefore, an example of endogenous formation of a free amino acid. The two-fold origin may be indicated in this manner:

Food Protein→cystin which in the presence of cholic acid yields
↗ taurine, especially taurocholic acid.
Liver Protein

In the case of N-minimum, however, cystin seems to be only locally set free, since none is found in the circulation. None can be held there with the aid of monobrombenzene and thus be brought as far as bromphenyl-



Brom-phenyl Mercapturic Acid

mercapturic acid. It is of no consequence whether brombenzene is administered by mouth and cystin into the intestine or *vice versa* (Kapfhammer, 1921). Cystin cannot be here replaced by any other sulphur-containing compounds (Muldoon, Shiple and Sherwin, 1924).

Intestinal putrefaction on a N minimum diet is greatly depressed through active carbohydrate fermentation. In spite of this, we find no

disappearance of the aromatic hydroxy acids in the urine. But, according to Abderhalden (1912), fed tyrosin is completely oxidized even in larger amounts. We have here an indication that tyrosin is not a step in the intermediary metabolism of the tyrosin complex in the tissues. We know the end products of the suprarenals and thyroid glands, epinephrin and thyroxin, and we know from the alcaptonuric that relatively very little homogentisic acid is derived from the endogenous protein metabolism (Katsch, 1918, 1920; Neubauer, 1929). It is possible, as Neubauer thinks, that since little tyrosin is present in such cases, that which is present is used for more important purposes; but it may also be assumed that the tyrosin is separated in a form which is no longer capable of being oxidized to homogentisic acid.

As a third building stone, I should like to introduce arginine. It also yields proofs that it is broken down differently when it comes from tissue protein than when it is fed in excess. Arginine is considered to be the mother substance of creatinine. According to the usual scheme of the breaking down of amino acids, the intermediate steps in its breakdown should be γ-guanidine-butyric acid and guanidine-acetic acid. Or in case the methylation takes place immediately we would have ∂-methyl-arginine and γ-methyl-guanidine-butyric acid. None of the aforementioned substances, however, yields creatinine in the animal body. The only probable one is guanadine-acetic acid which Jaffe has reported. But one must not conclude from this that it represents a normal intermediary product, for its methylation takes place only to a small extent, although the animal body is capable of methylating other substances foreign to it. Moreover, the guanidine acids are difficultly soluble substances and show an entirely different chemical behavior from arginine. For that reason Kapfhammer, Flaschentraeger and I (1922) developed the synthesis of ∂-methyl-arginine.

$H_2NC(NH)NHCH_2\text{–}COOH$

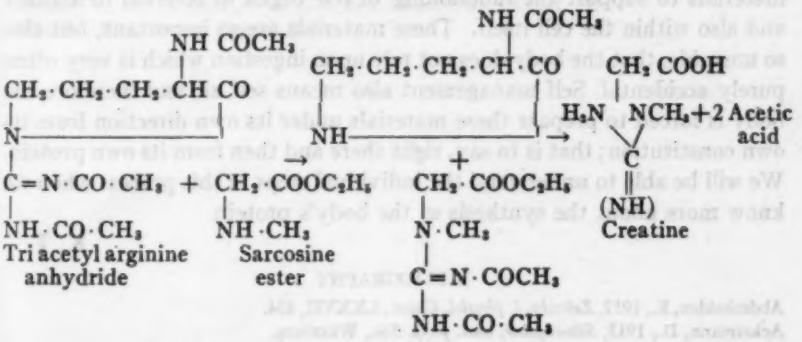
Guanido acetic acid

$H_2NC(NH)N(CH_3)CH_2 \cdot CH_2 \cdot CH_2 \cdot CH(NH_2)COOH$

∂–Methyl-arginine

The fact that this product also, which differs only so slightly from arginine, gave no creatin, surprised us more than that Thompson was able to prepare creatin from arginine and biological methylation agents. The methyl arginine was not all broken down but was found excreted in the urine completely unchanged. I conclude from this that the arginine behaves similarly to the aromatic amino acids. In their case the moiety

oxidation must take place before the breaking down at the alpha C atom. In the same manner, the arginase must first split the guanidine moiety so that then it is possible for the ornithine to go over into sugar in the liver perhaps by way of γ-amino-butyric acid (Felix and Tomita, 1923; Corley, 1926; Keil, 1927). The methyl group hinders the splitting action of the arginase. The further breaking down in the liver is consequently also depressed and the substance behaves as a foreign body, easily soluble, and is removed by way of the kidneys. But we have flooded the body with the methyl arginine and a portion of it must surely have succeeded in reaching the muscles. There it should, according to the usual hypothetical scheme, have been broken down oxidatively in the absence of arginase. That did not happen, however. I concluded from this that perhaps here, in accordance with the scheme of Hofmeister, only the side chain of the peptid-linked arginine was oxidized and that creatine resulted from the oxidation on the other end.



After this work was done, a period of several years elapsed before the beautiful reports of M. Bergmann (Bergmann and Köster, 1926; Bergmann and Zervas 1927) appeared. In the laboratory it is possible to loosen the guanidine moiety by means of acetylation; its cyanamid complex can then be transferred to other amines, including the ester of glycocoll or of sarcosine. In this manner, Bergmann obtained creatin.

According to Bergmann creatin has two mother substances arginine for the guanidine moiety and the precursors of glycin in the body, which are as yet unknown to us. In the liver, cleavage would take place by means of arginase, but the muscles do not possess this enzyme. Instead, the muscles have available an acylated arginine or creatin, in the form of phosphagen. These substances are constantly being regenerated. One

fact we can accept and that is that muscle arginine probably plays a part in the creatin formation but free arginine never.

In life all chemical reactions are catalyzed by the ferments, which are extraordinarily specific tools and are very definitely dependent upon the structure and configuration of the substrates. They require definite points for attack in order that the necessary intermediary product—the ferment-substrate complex may be formed. In the tissues within the cells we find different conditions, other relationships, other points of attack and other ferments. The cell is also capable of hydrolyzing its protein—that takes place in hunger or when the cell is mechanically destroyed or blood is removed, or in the autolysis of a pneumonic exudate. But the live cell does not have to hydrolyze and probably does not do it so far as that portion which involves the wear and tear quota is concerned. To be consumed means to be used, but not to be lost; if I may so express it. It is spent, after due consideration, for important, inevitably necessary purchases—for materials to support the functioning of one organ in relation to another and also within the cell itself. These materials are so important, but also so unstable, that the body does not rely upon ingestion which is very often purely accidental. Self management also means self aid and therefore the body is forced to prepare these materials under its own direction from its own constitution; that is to say, right there and then from its own protein. We will be able to understand the individual steps in this process when we know more about the synthesis of the body's protein.

K. T.

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